Brain plasticity and sleep in the course of development and after acquired brain injury

Thesis (cumulative thesis)

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TABLE OF CONTENTS

SUMMARY

Neural plasticity allows the brain to adapt its structure and function to internal and external changes. Such changes occur in the context of development, learning and training but also as a response to acquired brain injury (ABI) or in relation to rehabilitation therapy (Huttenlocher and Dabholkar, 1997; Giedd, 2004; Feldman, 2009; Murphy et al., 2009). It has been shown that plastic processes in the healthy brain are linked to changes in electroencephalographic slow wave activity during sleep (SWA, EEG spectral power 1-4.5 Hz, for a review see (Tononi and Cirelli, 2014)).

The aim of the present thesis was to investigate the relationship between sleep and plasticity in healthy children and adolescents and in children and adolescents with ABI. In healthy children and adolescents we found changes in the SWA topography in the course of development and after a specific learning-experience. In children and adolescents with ABI, we found lesion-related alterations in topographical SWA when compared to healthy children and adolescents. In children and adolescents with disorders of consciousness (DOC), the regulation of SWA was reduced over parietal brain areas when compared to healthy children and adolescents.

Overall, our results suggest that mapping SWA across the scalp is a promising approach to investigate brain maturation, experience-dependent plasticity and neural reorganization after ABI in children and adolescents.

ZUSAMMENFASSUNG

Neuronale Plastizität erlaubt dem Gehirn, sich in seiner Struktur und Funktion wechselnden externalen und internalen Einflüssen anzupassen. Solche Anpassungen erfolgen im Laufe der Entwicklung oder im Rahmen von Lern- und Übungsprozessen aber auch als Reaktion auf eine erworbene Hirnschädigung und im Zusammenhang mit Rehabilitationsmassnahmen (Huttenlocher and Dabholkar, 1997; Giedd, 2004; Feldman, 2009; Murphy et al., 2009). Bisherige Studien haben gezeigt, dass plastische Veränderungen mit einer erhöhten elektroenzephalografischen Tiefschlafaktivität (langsame Wellen im EEG: 1-4.5 Hz; siehe ein Review von (Tononi and Cirelli, 2014)) einhergehen.

Das Ziel der vorliegenden Dissertation war es, den Zusammenhang zwischen Schlaf und Plastizität bei gesunden Kindern und Jugendlichen und bei Kindern und Jugendlichen mit einer erworbenen Hirnschädigung zu untersuchen. Bei Gesunden fanden wir, dass sich die Tiefschlaftopografie sowohl im Verlauf der Entwicklung als auch nach einer bestimmten Lernerfahrung ändert. Bei Kindern und Jugendlichen mit einer erworbenen Hirnschädigung fanden wir verletzungsbedingte Veränderungen in der Tiefschlaftopographie. Bei Kindern und Jugendlichen mit beeinträchtigtem Bewusstsein war die Regulation der Tiefschlafaktivität über parietalen Hirnarealen vermindert.

Insgesamt legen unsere Resultate nahe, dass Tiefschlaftopografien eine vielversprechende Möglichkeit bieten um entwicklungs-, lern- und verletzungsbedingte neuronale Plastizität zu untersuchen.

1. INTRODUCTION

Neural plasticity allows the brain to adapt its structure and function to internal and external changes. Such changes occur in the context of development, learning and training but also as a response to acquired brain injury (ABI) or in relation to rehabilitation therapy (Huttenlocher and Dabholkar, 1997; Giedd, 2004; Feldman, 2009; Murphy et al., 2009). It has been shown that plastic processes in the healthy brain, namely, experience-dependent plasticity and brain maturation, are linked to sleep (Tononi and Cirelli, 2005; Diekelmann and Born, 2010; Huber and Born, 2014; Tononi and Cirelli, 2014). Specifically, electroencephalographic slow wave activity during deep sleep (SWA, EEG spectral power 1-4.5 Hz) is known to play a crucial role. Moreover, sleep is supposed to have a beneficial effect on learning and memory (Diekelmann and Born, 2010; Tononi and Cirelli, 2014). While a considerable amount of studies investigated the relationship between sleep and plasticity in healthy subjects, little is known about this relationship in the context of neural reorganization and function recovery after ABI. Since SWA seems to be a sensitive method to assess plastic processes in the healthy brain, it could also be used to investigate plasticity after ABI. Translating knowledge and methods from basic science into clinical research might provide novel insights into principles of neural reorganization.

In a first part of the introduction we will look at brain plasticity and underlying neural mechanisms in different contexts. The second part will introduce the basic principles of sleep and summarize what is known about the relationship between sleep and plasticity. The last part will present the aims of this theses.

1.1. Brain plasticity

1.1.1. Brain development

Post-natal brain development is associated with changes in synaptic density. In a first phase, synaptogenesis leads to a continuous increase in synapse density until the age of 1 to 2 years. This peak of synapse density is followed by synapse elimination, a process often referred to as "synaptic pruning". This second phase of brain development lasts until late adolescence (Huttenlocher and Dabholkar, 1997). Studies using magnetic resonance imaging (MRI) could show that consistent with histological findings, changes in cortical gray matter volume follow an inverted U-shape with an initial increase and a subsequent decrease. However, the time

course of this trajectory varies across different cortical regions (Giedd, 2004; Gogtay et al., 2004; Shaw et al., 2008; Group, 2012). Cortical gray matter maturation proceeds from posterior towards anterior regions i.e., occipital regions mature first and frontal regions last (Giedd, 2004; Gogtay et al., 2004; Shaw et al., 2008; Group, 2012). This fits the development of specific functions. While visual functions develop early in childhood, cognitive functions develop later (Chelune and Baer, 1986; Teller, 1990; Huizinga et al., 2006).

The development of functions is a stepwise process and has been linked to the concept of 'critical' or 'sensitive' periods. Such periods mark a time window during which specific neural networks are maximally receptive for specific learning-experiences (Hensch, 2004; Thomas and Johnson, 2008). For instance, early musical training results in more pronounced neural changes and better performance than musical training later in life (Steele et al., 2013). Studies investigating the critical period for first language acquisition found that children who were not appropriately exposed to language within the first year of life, later showed severe language impairments (for a review see (Friedmann and Rusou, 2015)). However, experience-dependent plasticity is not limited to critical periods. The training of specific functions later in life can also induces changes in neural networks and performance.

1.1.2. Experience-dependent plasticity

A crucial mechanism for experience-dependent plasticity is synaptic long-term potentiation or depression (Feldman, 2009). For instance, training a specific skill increases synaptic strength in brain areas involved in skill performance while a lack of practice results in a reduction of synaptic strength. MRI studies could show that the acquisition of new skills such as juggling or playing golf increases gray matter volume in task-relevant cortical regions (Draganski et al., 2004; Bezzola et al., 2011). Skill-specific changes in cortical gray matter were also found after foreign language acquisition and the practice of mirror reading (Ilg et al., 2008; Mårtensson et al., 2012). Another interesting study investigated the effect of unilateral arm immobilization (Langer et al., 2012). The lack of arm use was associated with a decrease in cortical gray matter volume in the contralateral motor area. Altogether, these results suggest that behavior can reshape brain structure. This potential is highly relevant for rehabilitation therapy in patients with acquired brain injury (ABI). It implies that training could re-establish disrupted neural networks and that inactivity should be avoided, since it would cause further losses in cortical gray matter. Following this line of thinking, a novel neurophysiological model of rehabilitation therapy has been proposed (Small et al., 2013). The model states that therapy should primarily be seen as a means of neural recovery, which then can lead to the

restoration of a function. In the next paragraphs we will look into plasticity in this context of neural reorganization.

1.1.3. Plasticity during stroke recovery

Beyond causing death of neurons, stroke disrupts functional networks of the brain, which might be essential for specific functions such as sensorimotor function, language or cognitive functions. The extent, to which functions can recover, mainly depends on the degree of tissue damage and the preservation of neural circuits engaging intact brain areas to restore function (Wieloch and Nikolich, 2006). Important key factors are redundant connections and the formation of new connections (Murphy and Corbett, 2009). It has been suggested that mechanisms underlying recovery are similar to those involved in plastic processes of the healthy brain, namely, brain development and experience-dependent plastic changes (Murphy and Corbett, 2009). Similar to 'critical' periods during early development, stoke is followed by a time window during which rehabilitation interventions have maximal effects on recovery (Biernaskie et al., 2004; Salter et al., 2006). These findings suggest that it is highly important to start rehabilitation therapy early.

Functional MRI studies investigating alterations in the pattern of brain activation in adult patients with stroke found that movements of the affected hand are associated with increased activity in contra-lesional motor and bilateral non-motor areas. Similar pattern of additional activity were also found for attention in patients with neglect and for language in patients with aphasia (for a review see (Grefkes and Ward, 2014)). These findings might reflect the formation of alternative networks. To date, no studies have investigated alterations in brain function in pediatric patients with stroke.

1.1.4. Altered network function after traumatic brain injury

Traumatic brain injury (TBI) can cause both focal cortical damage and axonal damage. Unlike peripheral nerves, which can regrow, axons of the brain do not regenerate (Bradke et al., 2012). Disconnected axons undergo Wallerian degeneration (Conforti et al., 2014). In contrast, demyelinated axons can recover function after myelin repair (Armstrong et al., 2016). However, the role of white matter in injury-related plasticity and functional recovery remains unclear.

Axonal injury in long-distance white matter tracts has been associated with large-scale network dysfunction (for a review see (Sharp et al., 2014)). For instance, patients with TBI showed an altered activation pattern of the default mode network during a cognitive task

which in turn was related to impaired performance. Such altered activation patterns have also been reported for other large-scale networks. However, changes were not always related to impaired performance, suggesting the presence of functional compensatory mechanisms (Sharp et al., 2014).

Alterations in brain activation have also been investigated in the pediatric patient population. During a motor coordination task, children with TBI showed an increased brain activation, when compared to typically developing children even though task performance was comparable between the two groups (Caeyenberghs et al., 2009). Similar results were also found for a working memory task (Westfall et al., 2015).

1.1.5. Brain function in patients with disorders of consciousness

Severe ABI, be it vascular or traumatic, can cause disorders of consciousness (DOC). DOC are categorized based on observable behavioral characteristics using assessment scales like the Coma Recovery Scale - Revised (Giacino et al., 2004). Patients in a coma are completely unarousable and unresponsive. The vegetative state (VS) or, using another currently proposed term the unresponsive wakefulness syndrome (UWS) (Laureys et al., 2010), is defined by the re-emergence of spontaneous eye-opening. Patients further evolving into a minimally conscious state (MCS), start to show non-reflexive responses to stimuli. The emergence from MCS is characterized by re-established functional communication or by the recovery of functional object use (Giacino et al., 2002). The assessment of patients with DOC is very challenging as a lack of motor functions, receptive aphasia or fluctuations in arousal might lead to false negative results and consequently to a misdiagnosis (Giacino et al., 2009). To overcome this issue various neuroimaging and electrophysiological studies have investigated neural correlates of consciousness (for reviews see (Laureys and Schiff, 2012; Schiff et al., 2014)). Using a wide range of findings as complementary pieces of the puzzle, a common network mechanism has been proposed (Giacino et al., 2014). The mesocircuit model (Figure 1) states that neural activity in the frontal cortex is reduced due to deafferentation and neuronal death. Further, the loss of neurons in the thalamus and impaired functional thalamocortical connectivity are supposed to result in an insufficient cortical activity preventing local processing and long-distance functional corticocortical connectivity. Finally, the reduced thalamostriatal and corticostriatal functional connectivity might fail to initiate active inhibition of the globus pallidus via striatum, which in turn causes an active inhibition of connected brain structures including the thalamus and possibly the pedunculopontine nucleus.

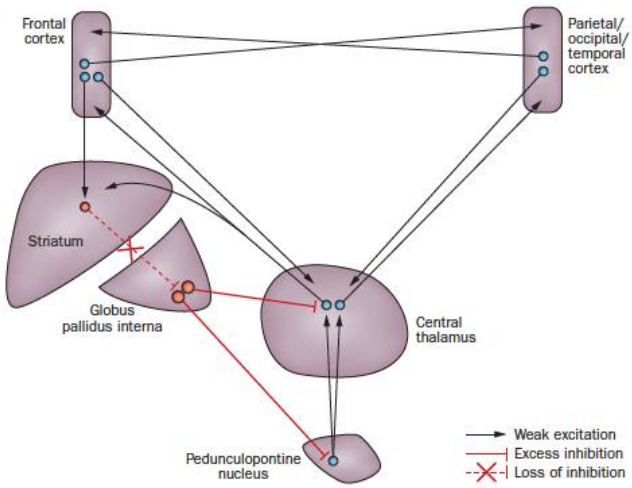

Figure 1 The mesocircuit model (Giacino et al., 2014)

Sleep studies have investigated brain activity during sleep in patients with DOC. The presence or absence of normal sleep elements such as different sleep stages, sleep spindles and sleep slow waves has been associated with behavioral diagnosis and/or outcome, and was hypothesized to reflect global functional brain integrity (Landsness et al., 2011; Cologan et al., 2012; Malinowska et al., 2013; de Biase et al., 2014; Forgacs et al., 2014; Kang et al., 2014; Rossi Sebastiano et al., 2014; Aricò et al., 2015; Arnaldi et al., 2015).

While, in the past, many complementary neurophysiological approaches to assess DOC have been investigated in adults, very few have been applied to children. In fact, only two studies reported more than single cases. Both investigated the presence or absence of sleep stages and sleep spindles, as a predictor for outcome (Cheliout-Heraut et al., 2002; Avantaggiato et al., 2015).

The next chapter will give an overview of basics in sleep research and elaborate how sleep and brain plasticity are related.

1.2. Sleep

Sleep is considered a restorative process that is crucial for effective cognitive function (Diekelmann and Born, 2010; Tononi and Cirelli, 2014). In particular, it has been shown that sleep has a beneficial effect on learning and memory (Diekelmann and Born, 2010; Tononi and Cirelli, 2014). On a neuronal level, sleep has been linked to synaptic plasticity, namely, experience-dependent plasticity and brain maturation (Tononi and Cirelli, 2005; Diekelmann and Born, 2010; Huber and Born, 2014; Tononi and Cirelli, 2014).

1.2.1. Sleep architecture

The patter of electroencephalographic (EEG) brain activity during sleep clearly differs from the pattern seen during wakefulness (Steriade et al., 1993b). Characteristic features of non-rapid-eye-movement (non-REM) sleep are slow waves, high amplitude waves below 4.5 Hz, and sleep spindles, waxing and waning oscillations between 12 and 15 Hz. The scoring manual from the American Academy of Sleep Medicine (AASM) provides criteria for determining sleep stages (Iber et al., 2007). Based on the EEG signal of brain activity, eye movements (measured by means of electrodes positioned close to the eyes) and muscle tone (measured by means of two chin electrodes) different vigilance stages are scored (wakefulness, non-REM sleep stages 1-3, REM sleep) (Figure 2, top). Episodes of non-REM and REM sleep show an alternating cyclic pattern throughout the night (Feinberg and Floyd, 1979). Deep sleep (non-REM 3) is most pronounced in the first sleep cycle and diminishes over subsequent cycles. REM-sleep in turn, shows the inverse pattern. The analysis of sleep slow waves, a characteristic feature of deep sleep, provides further insights into the regulation of sleep.

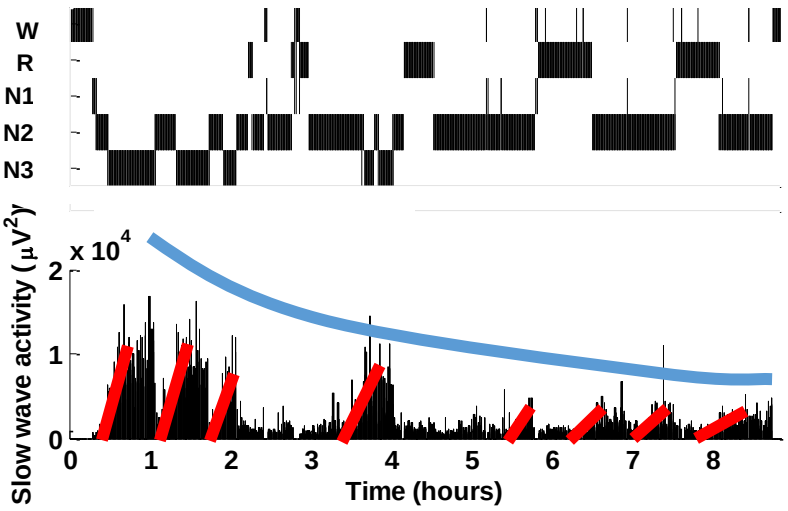

Figure 2 Hypnogram and the course of slow wave activity (SWA) across the night in a representative subject. The Hypnogram (top) shows the scored vigilance stages: wakefulness (W), non-REM sleep stages 1 to 3 (N1, N2, N3), REM sleep (R). The decline of SWA is indicated by the blue line, episodes of build-up SWA by red lines (bottom).

1.2.2. Sleep regulation

The activity of sleep slow waves (EEG spectral power 1-4.5 Hz) during non-REM sleep is a well-established quantitative measure for sleep depth (Borbely and Achermann, 2000). SWA is highest at the beginning of the night and declines towards the end of the night (Figure 2, bottom). This decline of SWA is supposed to reflect the dissipation of sleep pressure (Borbely

and Achermann, 2000; Tarokh et al., 2012). Another sensitive measure for sleep pressure is the build-up of SWA within non-REM sleep episodes (Figure 2, bottom). Accordingly, the build-up is fastest in episodes at the beginning of the night when sleep pressure is highest (Borbely and Achermann, 2000; Tarokh et al., 2012).

SWA is known to be regulated in a use-dependent manner (Figure 3). After prolonged wakefulness the amount of SWA is increased and the build-up of SWA within non-REM sleep episodes is faster (Borbely and Achermann, 2000; Jenni et al., 2005b). The synaptic homeostasis hypothesis provides a plausible explanation for these findings (Tononi and Cirelli, 2005, 2014).

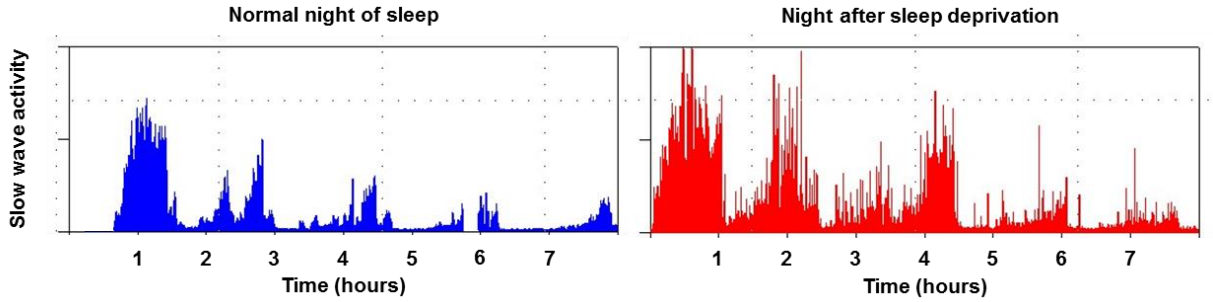

Figure 3 Slow wave activity (SWA) across the night in a normal night of sleep (left) and in a night after sleep deprivation (right). (Courtesy of Peter Achermann)

1.2.3. The synaptic homeostasis hypothesis

During wakefulness, when interacting with the environment, synaptic strength increases throughout the brain. However, synaptic strength cannot be increased infinitely. In order to regulate total energy consumption and total brain volume there is need for a process that reduces synaptic strength. The synaptic homeostasis hypothesis suggests that such a renormalization of synaptic strength occurs during slow wave sleep (Tononi and Cirelli, 2005, 2014). The authors state that "sleep is the price we pay for plasticity". Supporting evidence comes from a transcranial magnetic stimulation (TMS) study. Cortical excitability, measured by the neural response to TMS, continuously increased in the course of a day spent awake and returned to baseline level after a night of sleep (Huber et al., 2013). In the context of sleep deprivation, prolonged wakefulness results in excessive synaptic strength and an increased need for synaptic downscaling. This increased need for synaptic renormalization could explain the previously described increase in SWA after prolonged wakefulness (see paragraph 2.2.). Interestingly, use-dependent changes in SWA can also be induced locally.

1.2.4. Local SWA and plasticity

Several studies have investigated the effect of specific experiences on local sleep SWA. For instance, somatosensory stimulation to one hand resulted in a local increase in SWA over the contralateral somatosensory cortex (Kattler et al., 1994). The use of high-density EEG (up to 256 electrodes) provides the possibility to map SWA across the scalp. Such topographical maps can be used to investigate local changes in SWA. For instance, it has been demonstrated that a visuomotor learning task leads to an increase in sleep SWA over brain areas known to be involved in task performance (Huber et al., 2004). The fact that specific experiences locally increase SWA, further supports the assumption that synaptic plasticity and SWA are linked. Furthermore, one day of unilateral arm immobilization resulted in a decrease in SWA over the contralateral somatosensory cortex (Huber et al., 2006). Thus, reduced synaptic strength requires less synaptic down regulation during sleep.

Since SWA presumably reflects experience-dependent plasticity, the question arises whether SWA can also track maturational changes in the course of development.

1.2.5. Maturational changes in SWA

In the course of development the expression of sleep slow waves changes substantially. SWA increases over the first years of life with a peak shortly before puberty and a subsequent decline throughout adolescence (Campbell and Feinberg, 2009). This trajectory parallels the course of synapse density and cortical gray matter thickness (Huttenlocher and Dabholkar, 1997; Giedd, 2004). The topographical distribution of SWA also shows age-dependent changes. From early childhood to late adolescence the location of maximal SWA shifts from posterior towards anterior brain regions (Kurth et al., 2010a). These changes fits the pattern of cortical gray matter maturation, which is known to proceed from occipital to frontal brain regions (Shaw et al., 2008). A study combining sleep EEG recordings and MRI data in children and adolescents could show that indeed, SWA is a suitable marker for age-dependent changes in cortical gray matter (Buchmann et al., 2011). Moreover, maturational changes in the topographical distribution of SWA have been associated with the development of specific skills (Kurth et al., 2012).

1.3. Addressed open questions

The global aim of the present thesis was to investigate the relationship between sleep and plasticity in healthy children and adolescents and in children and adolescents with ABI. We pursued a translational approach. We used basic science results from the healthy population and developed approaches to investigate the clinical population.

1) The topographical distribution of sleep SWA shows age-dependent state-like aspects that have been related to cortical gray matter maturation and the development of specific skills (Kurth et al., 2010a; Buchmann et al., 2011; Kurth et al., 2012). Repeated measurements in adults as well as in children and adolescents showed that SWA topographies are highly consistent within subjects, which indicates trait-like aspects in the topographical distribution of SWA (Finelli et al., 2001b; Lustenberger and Huber, 2012). Do longitudinal measurements in the course of development show both, state- and trait-like aspects?

   Research article:

   Individual Slow Wave Activity Trajectories as a Marker for Brain Development

   Lustenberger, C., Mouthon, A-L., Tesler, N., Kurth, S., Ringli, M., Pugin, F., Huber, R.

   Submitted

2) In adults, experience-dependent plasticity is related to local changes in sleep SWA (Huber et al., 2004). Can this relationship also be seen in typically developing children and adolescents?

   Research article:

   Sleep Slow-Wave Activity Reveals Developmental Changes in Experience-Dependent Plasticity

   Ines Wilhelm, Salome Kurth, Maya Ringli, Anne-Laure Mouthon, Andreas Buchmann, Anja Geiger, Oskar G. Jenni, and Reto Huber

   Published: The Journal of Neuroscience, September 10, 2014 • 34(37):12568 –12575

3) SWA during sleep reflects plastic processes in the healthy brain (Huber et al., 2004; Campbell and Feinberg, 2009; Kurth et al., 2010a). Does SWA also indicate neural plasticity after ABI?

Research article:

Sleep slow wave activity: towards a new marker for neural plasticity after acquired brain injury

Anne-Laure Mouthon, MSc; Hubertus J.A. van Hedel, Andreas Meyer-Heim, MD; Salome Kurth, PhD; Maya Ringli, PhD; Fiona Pugin, PhD; PhD; Reto Huber, PhD

Submitted

4) SWA sleep is tightly related to previous wakefulness and is regulated in a use-dependent manner (Kattler et al., 1994; Huber et al., 2006). Can SWA serve as an indirect measure for brain function during previous wakefulness in patients with DOC? And could local alterations in the sleep topography indicate brain areas playing a crucial role in brain network dysfunction underlying DOC?

Research article:

High-density electroencephalographic recordings during sleep in children with disorders of consciousness

Anne-Laure Mouthon, Hubertus J.A. van Hedel, Andreas Meyer-Heim, Salome Kurth, Maya Ringli, Fiona Pugin, Reto Huber

Under review

The following paper section starts with a review article about methods in pediatric sleep research and sleep medicine. The section then continues with the above listed research articles.

2. ARTICLES

2.1. Methods in pediatric sleep research and sleep medicine

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Published: Neuropediatrics. 2015 Jun;46(3):159-70.

Abstract

Several methods are used to evaluate sleep in infants, children and adolescents: Questionnaires and diaries, actigraphy, polysomnography and electroencephalography are well established. Novel approaches such as high-density electroencephalography, simultaneous electroencephalography - functional magnetic resonance imaging and non-pharmacological methods aiming for a modulation of sleep are currently only used for research. These approaches might become valuable methods for clinical application in the future. The purpose of this review is to present an overview of current methods and their respective fields of application and to report available rules and recommendations for their use.

Introduction

In pediatric sleep medicine clinicians assess sleep to identify sleep problems and to diagnose sleep disorders. Sleep problems such as bedtime problems, night wakings, and poor sleep hygiene are highly prevalent in the pediatric population. It has been reported that approximately 25% of all children experience some type of sleep problem at least once during childhood (Owens, 2011) however, sleep disorder diagnoses are less common. Pediatric sleep disorders include sleep-related breathing disorders (prevalence: 4-11% (Lumeng and Chervin, 2008)), obstructive sleep apnea (prevalence: 1-4% (Lumeng and Chervin, 2008)), restless legs syndrome (prevalence: 2% (Picchietti et al., 2007)) periodic limb movement disorder (prevalence: 14% (Gingras et al., 2011)), narcolepsy (prevalence: 0.05% (Peterson and Husain, 2008)), insomnia (20-30% (Owens and Mindell, 2011)), and parasomnias (prevalence: 14.4% (Agargun et al., 2004)).

Sleep researchers assess pediatric sleep in order to investigate developmental changes in sleep behavior and neurobiological sleep characteristics. Clinical research aims at identifying discrepancies between clinical populations and typically developing children and adolescents.

Several methods have been developed to cover the needs of clinicians and researchers. The methods differ in terms of information source (objective vs. subjective), time and financial costs and setting (sleep lab vs. habitual environment). Accordingly, they all have their specific field of application.

Questionnaires and diaries

In a review from 2011 the authors evaluated currently used questionnaires and scales about sleep in children (Spruyt and Gozal, 2010). They found 57 instruments in which psychometric testing had been done to some extent. Best ratings for instruments assessing sleep problems in infants (1 month to 2 years) were obtained by the Sleep and Settle Questionnaire (SSQ), the Maternal Cognitions about Infant Sleep Questionnaire (MCISQ) and the Parental Interactive Bedtime Behavior Scale (PIBBS). These instruments mainly focus on sleep environment and settling. In children (2 to 11 years) the instruments focus more on sleep-wake patterns, routines, sleep hygiene and the screening for specific sleep disorders like insomnia, sleep-related breathing disorders or periodic limb movement disorder. Towards adolescence (11 to 18 years) more questions relating to sleepiness or emotional well-being are included. The authors recommend the use of the Bedtime Routines Questionnaire (BRQ), the Tayside

Children's Sleep Questionnaire (TCSQ), the Children's Sleep Wake Scale (CSWS), the Behavioral Evaluation of Disorders of Sleep Scale (BEDS), the Pediatric Sleep Questionnaire (PSQ), the Sleep-related Breathing Disorders Scale (SRBD), the Sleep Disturbance Scale for Children (SDSC) and the Sleep Disorders Inventory for Students – Children (SDIS-C). The latter disposes of a specific version for adolescents (SIDIS-A). The Dream Content Questionnaire for Children (ChDCQ) and the Cleveland Adolescent Sleepiness Questionnaire (CASQ) were the only self-report instruments with good ratings. A recent preliminary study showed good psychometric values for a newly developed self-report tool for children (Meltzer et al., 2013): the Children's Report of Sleep Patterns (CRSP). The authors claim that such self-reports might provide complementary information that would not be covered if only relying on parental reports.

Using these instruments, in several clinical populations the prevalence for sleep disorders was found to be increased when compared to the healthy population i.e., in children and adolescents with attention-deficit/hyperactivity disorder (ADHD), in children and adolescents with autistic spectrum disorder (ASD), in children and adolescents with cerebral palsy and in children and adolescents with down syndrome (e.g., (Moreau et al., 2013; Hodge et al., 2014; Hoffmire et al., 2014; Romeo et al., 2014)). The most commonly used instruments to screen for sleep disorders in these children are the Children's Sleep Habit Questionnaire (CSHQ), the Sleep Disturbance Scale for Children (SDSC) and the Pediatric Sleep Questionnaire (PSQ). The Sleep Self-report (SSR) for children and adolescents is mainly used in combination with the CSHQ for parents (e.g., (Owens et al., 2000; Sumpter et al., 2013)). The Questionnaire for Children with Severe Psychomotor Impairment (Schlaffragebogen für Kinder mit Neurologischen und Anderen Komplexen Erkrankungen, SNAKE) is a recently developed instrument to assess sleep disorders in children and adolescents with severe psychomotor impairments (Blankenburg et al., 2013). It specifically takes into account impaired perception, intellectual disability and motor impairment. Another instrument aiming at a specific patient group is the Pediatric Restless Legs Syndrome Severity Scale (P-RLS-SS, (Arbuckle et al., 2010)). However, the scale has not yet been validated.

While questionnaires and scales ask parents or children to reflect on weekly or monthly sleep behavior, diaries require a daily report of sleep and wake phases. Such diary-based reports were found to be a reliable source of information for sleep start, sleep end and assumed sleep but not for nocturnal wake time when compared to objective measurements assessed by actigraphy (Werner et al., 2008). In children with sleep disorders this discrepancy

between parental report about nocturnal wake time and actigraphy seems to be even more pronounced (Werner et al., 2014).

Actigraphy

Actigraphy uses a watch-like movement sensor to assess habitual sleep-wake patterns. It allows data collection over multiple days and is easily applied in the child's natural environment. At least five nights are required to obtain reliable measures (Acebo et al., 1999). The most commonly used devices are the AMI devices (Ambulatory Monitoring Inc. actigraphs: Ardsley, New York), the Mini-Mitter devices (now owned by Phillips-Respironics, Bend, Oregon) and the Cambridge Actiwatch actigraphs (Cambridge, UK). Across all devices epoch length is most frequently set at 1 minute, less often at 30 seconds (Meltzer et al., 2012). Sleep-wake scoring algorithms respectively wake threshold sensitivity typically are device-specific. According to Meltzer et al. (Meltzer et al., 2012), the most commonly used sleep-wake scoring algorithm for the AMI devices is the Sadeh algorithm (Sadeh et al., 1991). For the Mini-Mitter and the Cambridge devices the most commonly used wake threshold sensitivity level is the medium sensitivity. The authors suggest that since sleep undergoes major changes in the course of development, devices and scoring algorithms / sensitivity levels should be selected age-specifically, based on previously published validation studies. They list 10 validation studies for different age groups which compared actigraphy to "gold standard" sleep measures like polysomnography. A more recent validation study used different devices and scoring algorithms in children and adolescents (Meltzer et al., 2012). Another recent study tested different wake threshold sensitivity levels specifically in 2 to 5 years old children (Belanger et al., 2013). Throughout all age groups, devices, epoch lengths, and scoring algorithms, studies consistently reported high sensitivity (proportion of correctly identified sleep epochs) and low specificity (proportion of correctly identified wake epochs). Thus, actigraphy accurately scores sleep periods, but is less suitable for detecting wake periods after sleep onset.

Actigraphy sleep variables such as sleep onset, wake after sleep onset and sleep offset are determined according to time-related definitions. For example, sleep onset is commonly defined by a number of consecutive epochs scored as sleep. However, there are no standards for such definitions. To address this concern, Meltzer et al. (Meltzer et al., 2012) provided a list of recommended variable names and definitions that should be considered when reporting results from actigraphy measurements (table1). Variables like bedtime and wake time are

assessed using actigraphy markers (button press) or daily sleep logs (table 1). Furthermore, sleep logs are needed to determine artifacts like sleeping in a car or times when the device is removed. Actigraphy has become a widely used method to objectively measure sleep over the last 20 years and has proven to be useful in assessing habitual sleep pattern in children with and without sleep problems (Werner et al., 2008; Werner et al., 2014). In clinical research, actigraphy is used to investigate sleep and the relationship between sleep and behavioral functions in different clinical populations e.g., children with ADHD (Lee et al., 2014) or children with Down syndrome or Williams syndrome (Ashworth et al., 2013). In children and adolescents with neurodevelopmental disorders the method allows to detect effects of medication on sleep (e.g., (Malow et al., 2011; De Crescenzo et al., 2013)). However, actigraphy is not a suitable method for the diagnosis of disorders in which sleep is fragmented. For example, the detection of limb movement events in children and adolescents with periodic limb movement disorder is insufficiently accurate (Montgomery-Downs et al., 2005). And in children and adolescents with obstructive sleep apnea actigraphy fails to reliably identify breathing abnormalities (O'Driscoll et al., 2009). For such clinical populations polysomnography remains the best diagnostic method.

Table1: Recommended variable names and definitions for actigraphy in the pediatric population

Reported variables	
Bedtime	Clock time attempted to fall asleep as indicated by either sleep log or event marker
Wake time	Clock time of final awakening in the morning as indicated by either sleep log or event marker
Sleep opportunity (Time in bed)	Time between bedtime and wake time (reported in min or h)
Actygraphy variables	
Sleep onset	Clock time for first of a predetermined number of consecutive minutes of sleep following reported bedtime
Sleep offset	Clock time for last of a predetermined number of consecutive minutes of sleep prior to reported wake time
Sleep period	Time between sleep onset and sleep offset (reported in min or h)
Total sleep time (TST)	Duration of sleep in sleep period (reported in min or h)
Sleep onset latency	Time between bedtime and sleep onset (reported in min)
Wake after sleep onset (WASO)	Number of minutes scored as wake during sleep period
Sleep efficiency	Percentage sleep: (TST / time in bed) x 100
Night waking	Predetermined minimal number of minutes of wake (e.g., >5 min) preceeded and followed by a predetermined minimal number of minutes of sleep (e.g., >15 min)
Night waking frequency	Number of night wakings
Night waking duration	Sum of minutes scored as night waking
24 h sleep duration	Amount of sleep in a 24 h period (reported in min or h)

Note: Adapted from Meltzer et al., 2012

Polysomnogrphaphy

The American Academy of Sleep Medicine (AASM) manual for the Scoring of Sleep and Associated Events provides technical specifications for polysomnography (PSG) recordings and criteria for determining sleep stages, arousals, respiratory events, cardiac events and movement events (Iber et al., 2007). According to these international guidelines, the electroencephalogram (EEG) should include at least eight electrodes, placed according to the international 10-20 system: bilateral frontal (F4, F3), central (C4, C3), occipital (O2, O1) and mastoids (M1, M2). Electrooculogram (EOG) is recorded using two electrodes (placed 0.5-1 cm above the right outer canthus and 0.5-1 cm below the left outer canthus, depending on the children's head size). Electromyogram (EMG) is recorded using submental electrodes. Based on these parameters sleep stages are scored (wakefulness, non-rapid-eye-movement sleep stages 1-3, rapid-eye-movement sleep). The 2007 AASM manual specifies scoring rules for children. Recommended sleep variables are listed in table 2. The scoring rules for sleep arousals are the same for adults and children. The number of arousals and the arousal index are the most currently used variables to quantify sleep disruption (table 2). Alternative measures, such as sleep pressure score, cyclic alternating pattern or computer-assisted identification of non-visible arousals may provide complementary information (Paruthi and Chervin, 2010). For the respiratory monitoring during PSG, the 2007 AASM manual recommends to measure a) airflow using an oronasal thermal sensor and a nasal air pressure transducer, b) respiratory effort using esophageal manometry or respiratory inductance plethysmography, c) oxygen saturation using pulse oxymetry and d) hypoventilation using transcutaneous or end-tidal PCO2 monitoring. In 2012 the AASM Sleep Apnea Definitions Task Force reviewed evidence for new monitoring technologies and further recommend the use of positive airway pressure (PAP) device flow signal for PAP titration PSG and the use of arterial PCO2 monitoring for hypoventilation (Berry et al., 2012). To detect snoring they recommend several sensors as options: acoustic sensor (e.g., microphone), piezoelectric sensor or nasal pressure transducer. The 2007 AASM manual provides scoring rules for respiratory events such as obstructive apnea, mixed apnea, central apnea, hypopnea, respiratory effort related arousals, hypoventilation and periodic breathing. All scoring rules are specified for children. The 2012 update of the AASM manual (Berry et al., 2012) adapted the pediatric scoring rules for central apnea and hypopnea (table 3), thereby improving the detection of sleep disordered breathing in children when compared to previous standards (Lin

and Guilleminault, 2011; Nixon et al., 2014). Recommended respiratory variables are listed in table 2.

PSG recordings also include an electrocardiogram (ECG). The 2007 AASM manual recommends the use of a 2-lead electrocardiograph with electrodes placed on the torso. Scoring rules are the same in adults and in children. Cardiac variables are listed in table 2. According to the 2007 AASM manual the leg electromyogram (EMG) should be recorded using surface electrodes placed longitudinally and symmetrically around the middle of the anterior tibialis muscle so that they are 2 to 3 cm apart or 1/3 of the length of the muscle, whichever is shorter. Both legs should be monitored for the presence of leg movements, preferably using separate channels for each leg. Recommended movement variables are listed in table 2.

Indications for PSG in the pediatric population are: 1) diagnosis of obstructive sleep apnea (OSA), 2) clinical evaluation after OSA treatment, 3) diagnosis of periodic limb movement disorder (PLMD) and 4) diagnosis of narcolepsy (Aurora et al., 2011; Aurora et al., 2012).

According to the American Academy of Pediatrics (AAP) PSG is the current gold standard for the diagnosis of pediatric OSA (Marcus et al., 2012). The apnea hypopnea index (AHI) is a commonly used to quantify OSA severity. However, there is no consensus in terms of AHI cutoff values. The current practice is to use an arbitrary cutoff >3 standard deviations beyond the mean of the normative AHI (Tan et al., 2013). Such normative values have been provided for infants, children and adolescents (Scholle et al., 2011; Brockmann et al., 2013). A recent study investigated whether results obtained with respiratory polygraphy (RP) or PSG are comparable. Although RP would be simpler and more cost-effective, the AHI is underestimated when compared to PSG, notably in children with mild and moderate OSA (Tan et al., 2014). Novel approaches propose the use of algorithms for therapy indication. In addition to parameters derived from PSG such algorithms include factors like the severity of symptoms, risk factors, and the presence of any OSA-related morbidity (Gozal and Kheirandish-Gozal, 2010; Kaditis et al., 2012). Current treatments of pediatric OSA are adenotonsillectomy, positive airway pressure (CPAP or BiPAP), high flow nasal cannula oxygen therapy and administration of anti-inflammatory agents such as montelukast or nasal budesonide (Tan et al., 2013), all significantly reducing the AHI. Treatment effects have been evaluated with follow-up PSG and PAP titration PSG (Marcus et al., 2006; Kheirandish-Gozal and Gozal, 2008; McGinley et al., 2009; Goldbart et al., 2012; Marcus et al., 2013).

According to the AASM international classification of sleep disorders, the diagnosis of periodic limb movement disorder (PLMD) requires PSG recordings. One of the diagnostic

criteria is a periodic limb movements of sleep index (PLMSI) >5/h (Medicine, 2005). Normative data support the clinical periodic limb movement index cutoff of > 5/h (Marcus et al., 2014). Periodic limb movements during sleep were found to be infrequent in the typically developing children and adolescents. Positive treatment effects of oral or intravenous iron on pediatric PLMD are found in 60-70% of the cases (Simakajornboon et al., 2003; Grim et al., 2013). The diagnosis of restless legs syndrome (RLS) in children is challenging, particularly because many young children are unable to describe typical RLS symptoms. Although not essential for diagnosis, a PLMSI >5 per hour is considered supportive evidence (Picchietti et al., 2013). In children diagnosed with RLS a PLMSI >5 per hour has been found in 63–74% of the cases (Kotagal and Silber, 2004; Muhle et al., 2008; Picchietti et al., 2009).

As part of the diagnostic evaluation in patients with narcolepsy the Multiple Sleep Latency Test (MSLT) is performed. This test assesses sleep latency and sleep onset rapid eye movement sleep periods (SOREMPs) for 4 to 5 daytime naps. A mean sleep latency <8 min and 2 or more SOREMPs is considered the cutoff for narcolepsy diagnosis (Medicine, 2005). However, there are no specifications for children. Overnight PSG is systematically performed prior to MSLT, primarily to rule out other causes of excessive daytime sleepiness. Recent studies in adults and children propose to use night PSG for diagnosis (Andlauer et al., 2013; Reiter et al., 2014). The authors suggest short REM sleep latency or SOREMP to be diagnostic for narcolepsy. In the absence of such findings, however, subsequent MSLT would still be required.

In clinical research, PSG is used to investigate sleep and the relationship between sleep and behavioral functions in different patient populations. E.g., children with ADHD were found to have a higher arousal index and a higher PLMSI (Ferri et al., 2013). In children with Down Syndrome and comorbid OSA cognitive performance was significantly lower than in those without OSA (Breslin et al., 2014). Increased sleep onset latencies and reduced REM sleep latencies were found in children and adolescents with depressive disorders (Lofthouse et al., 2009) as well as in children with generalized anxiety disorder (Alfano et al., 2012).

For many research questions comprehensive PSG is not needed. When respiratory and movement parameters are not involved, EEG recordings are sufficient.

Table2: Recommended variable names and definitions for polysomnography in the pediatric population

A Sleep variables	
1 Lights out	Clock time
2 Lights on	Clock time
3 Total sleep time (TST)	(in min)
4 Total recording time	Time between lights out and lights on (in min)
5 Sleep latency (SL)	Time between lights out and first epoch of sleep (in min)
6 REM sleep latency	Time between first epoch of sleep and first epoch of REM sleep (in min)
7 Wake after sleep onset (WASO)	Wake time during A4 - A5 (in min)
8 Sleep efficiency	Percentage sleep: (A3 / A4) x 100
9 Sum of sleep time for each sleep stage	(in min)
10 Percentage of TST for each sleep stage	(A9 / A3) x 100
B Arousal variables	
1 Number of arousals	
2 Arousal index (ArI)	(B1 x 60 / A3)
C Respiratory variables	
1 Number of obstructive apneas	
2 Number of mixed apneas	
3 Number of central apneas	
4 Number of hypopneas	
5 Number of apneas + hypopneas	
6 Apnea index (AI)	(C1 + C2 + C3) x 60 / A3
7 Hypopnea index (HI)	C4 x 60 / A3
8 Apnea + Hypopnea index (AHI)	C5 x 60 / A3
9 Continuous oxygen saturation	Mean value
10 Minimum oxygen saturation during sleep	
11 Occurence of Cheyne Stokes breathing	(yes / no)
D Cardiac variables	
1 Average heart rate during sleep	
2 Highest heart rate during sleep	
3 Highest heart rate during recording	
4 Bradycardia	(yes / no), if present report lowest heart rate observed
5 Asystole	(yes / no), if present report longest pause observed
6 Sinus tachycardia during sleep	(yes / no), if present report highest heart rate observed
7 Narrow complex tachycardia	(yes / no), if present report highest heart rate observed
8 Wide complex tachycardia	(yes / no), if present report highest heart rate observed
9 Atrial fibrilation	(yes / no)
10 Other arrhythmias	(yes / no), if present list arrhythmia
E Movement variables	
1 Number of periodic limb movements of sleep (PLMS)	
2 Number of periodic limb movement of sleep (PLMS) with arousals	
3 PLMS index (PLMSI)	E1 x 60 / A3
4 PLMS arousal index (PLMSArI)	E2 x 60 / A3

Electroencephalography

In basic and clinical research several sleep EEG measures have been assessed in the course of development. Discrepancies from age norms might be indicative for neurodevelopmental disorders. For example, the relative proportion of NREMS and REMS changes in the course of development (Feinberg et al., 2011b). The percentage of REMS increases from childhood to adolescence. In children and adolescents with autistic spectrum disorder (ASD) the percentage of REMS was found to be significantly lower when compared to typically developing children and adolescents of the same age (Buckley et al., 2010).

Sleep slow waves during NREMS are a well established marker for deep sleep. They are generated and maintained by thalamocortical and corticocortical networks (Steriade, 2003). The activity of these slow waves (slow wave activity, SWA: spectral power 1-4.5 Hz) is known to be regulated in a use-dependent manner i.e., SWA is increased after prolonged wakefulness in adults (Achermann and Borbely, 1990) as well as in children and adolescents (Kurth et al., 2010b). In the course of development the expression of slow waves changes substantially. SWA is known to increase over the first years of life with a peak shortly before puberty and a subsequent decline throughout adolescence (Jenni and Carskadon, 2004; Campbell and Feinberg, 2009).

The decay of SWA across the night has been used as a measure for the dissipation of sleep pressure in adults as well as in children and adolescents (Achermann and Borbely, 1990; Jenni and Carskadon, 2004; Tarokh et al., 2012).

Another sleep measure is the slope of sleep slow waves which has been proposed to reflect neuronal synchronization in adults (Riedner et al., 2008), in children and adolescents (Kurth et al., 2010b) and in infants (Fattinger et al., 2014). An overnight decrease in the slope of slow waves was shown to be already present in infants (Fattinger et al., 2014). In children with continuous spikes and waves during slow wave sleep (CSWS) the absence of this overnight decrease was suggested to reflect non-restorative sleep (Bolsterli et al., 2011) and to be related to neuropsychological deficits in these children (Bolsterli Heinzle et al., 2014).

Sleep spindels are a characteristic feature of NREMS stage 2 and have been described as waxing and waining oscillations between 12 and 15 Hz. Like slow waves they are known to be related to thalamocortical and corticocortical network activity (Steriade, 2003). In the course of development sleep spindle activity changes in terms of frequency, amplitude, length, and density (Jenni and Carskadon, 2004; Scholle et al., 2007). In adults as well as in

children and adolescents sleep spindels have been related to cognitive abilities (Schabus et al., 2006; Geiger et al., 2011a; Chatburn et al., 2013; Hoedlmoser et al., 2014).

Sleep characteristics can not only be investigated globally. Interstingly, sleep regulation also shows local, experience-related changes. For example, after unilateral sensory stimulation SWA at the corresponding central electrode site over the sensorymotor cortex was found to be higher when compared to the contralateral electrode site (Kattler et al., 1994). Frontal slow oscillations (SO: spectral power < 1 Hz) were found to be related to declarative memory consolidation (Diekelmann and Born, 2010). Recent studies investigating sleep and memory in children could show that frontal SO are correlated with declarative and emotional memory performance in typically developing children, but not in children with ADHD (Prehn-Kristensen et al., 2011; Prehn-Kristensen et al., 2013).

Another measure using local information from specific electrode sites is EEG coherence. Coherence measures are supposed to reflect brain connectivity. EEG signals are correlated between two recording sites from the same hemisphere (intrahemispheric coherence) or from distinct hemispheres (interhemispheric coherence) (Achermann and Borbely, 1998). A high correlation of neural activity between two recording sites indicates that those regions are directly connected or are both connected to a common third region. Developmental changes in coherence have been assessed from early childhood to adolescence (Tarokh et al., 2010; Kurth et al., 2014) and were suggested to reflect white matter brain maturation. In adolescents changes in intra-hemispheric coherence have been related to improved cognitive abilities (Tarokh et al., 2014). Alterations in coherence were found in children, adolescents and young adults with ASD. Studies found a reduction in intra-hemisperic fronto-central coherence and an increase in intra-hemispheric left occipito-parietal and occcipito-frontal coherence (Lazar et al., 2010; Leveille et al., 2010). In children and adolescents with major depressive disorder both, intra- and inter-hemisperic coherence was found to be reduced when compared to typically developing children and adolescents (Armitage et al., 2006). In a recent study the authors calculated coherence values over 19 electrodes (placed according to the 10-20 international system) in infants, children and adolescents, thereby obtaining topographical coherence maps for different age groups (Chu et al., 2014). They proposed the coherence maps to represent neuronal network maturation.

Mapping EEG measures over the scalp requires a larger number of electrodes than commonly used for sleep EEG recordings. High-density EEG (hdEEG) uses up to 256 electrodes.

Table3: Recommended changes to the AASM pediatric respiratory scoring rules

Scoring rules for pediatric apnea
Score a respiratory event as an apnea if it meets all of the following criteria: 1. There is a drop in the peak signal excursion by ≥ 90% of the pre-event baseline. 2. The duration of the ≥ 90% drop lasts at least the minimum duration as specified by obstructive, mixed, or central apnea duration criteria.
Scoring rules for pediatric central apnea
Score a respiratory event as central apnea if it meets apnea criteria, is associated with absent inspiratory effort throughout the entire duration of the event, and at least on of the following criteria is met: 1. The event lasts 20 seconds or longer. 2. The event lasts at least the duration of two breaths during baseline breathing and is associated with an arousal or ≥ 3% oxygen desaturation. 3. For infants younger than 1 year of age, the event lasts at least the duration of two breaths during baseline breathing and is associated with a decrease in heart rate to less than 50 beats per minute for at least 5 seconds or less than 60 beats per minute for at least 15 seconds.
Scoring rules for pediatric hypopnea
Score a respiratory event as a hypopnea if it meets all of the following criteria: 1. The peak signal excursions drop by ≥ 30% of pre-events baseline. 2. The duration of the ≥ 30% drop lasts for at least 2 breaths. 3. There is ≥ 3% desaturation from pre-event baseline or the event is associated with an arousal.

Note: Adapted from Berry et al., 2012

High-density electroencephalography

The high number of electrodes opens up entirely new possibilities of EEG signal analysis. Mapping the EEG activity at each electrode creates a topographical picture, visualizing the EEG activity distribution over the scalp. For example, investigating age-related differences in the topographical distribution of SWA revealed an interesting developmental trajectory (Figure 1): From early childhood to late adolescence the location of maximal SWA undergoes a shift from posterior towards anterior brain regions (Kurth et al., 2010a). This pattern corresponds to the course of cortical gray matter maturation. Thus, the SWA topography seems to be a marker for the maturational state of the brain. The course of developmental changes in the SWA topography has been related to skill maturation (Kurth et al., 2012) and showed local gender-specific differences (Ringli et al., 2013). This mapping tool might be promising to assess regional differences in brain activity in clinical populations. For example, mapping SWA in children with an Attention-Deficit Hyperactivity Disorder (ADHD) revealed increased SWA over central brain regions when compared to typically developing children and adolescents (Ringli et al., 2012). This pattern of SWA distribution in ADHD patients has been hypothesized to reflect altered or delayed brain maturation. Finally, the topographical distribution of EEG activity in other frequency ranges was also investigated. For example, a study investigated the topographical distribution of sleep spindel activity in children and adolescents (Geiger et al., 2011b). The authors found region-specific positive correlations between spindel activity and cognitive abilities.

Hd EEG can also be used to investigate task related local changes in brain activity. For example, studies have investigated experience-dependent changes in SWA in adults (Huber et al., 2004) and more recently in children and adolescents compared to adults (Wilhelm et al., 2014). Interestingly, the task related local increase of SWA was highest in children, suggesting a critical period of higher neuronal sensitivity to experience when compared to adolescents and adults. An experience-dependent increase in SWA was also shown after 3 weeks of working memory training in children and adolescents (Pugin et al., 2015).

An even higher spatial resolution of sleep brain activity including deep subcortical structures like for example, the thalamus, can be obtained by simultaneous EEG and functional magnetic resonance imaging (fMRI).

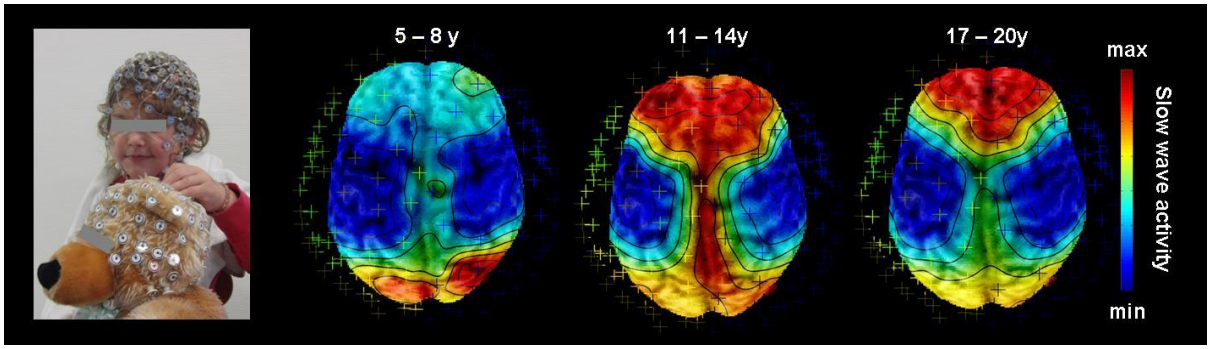

Figure 1 left: 4-year-old girl wearing a high-density EEG net (128 channels; Electrical Geodesics Inc.) and teddy bear wearing a training net. right: Topographical maps of relative sleep electroencephalography (EEG) slow wave activity (1-4.5 Hz) for different age groups superimposed over T1-weighted magnetic resonance images. Crosses surrounding the brain illustrate registered electrode positions on the scalp. Slow wave activity is colour coded (maxima in red, minima in blue). Values between electrodes were interpolated.

Electroencephalography - functional magnetic resonance imaging

EEG-fMRI combines EEG information such as sleep stages or sleep features (e.g., slow waves or sleep spindels) with fMRI network connectivity measures, i.e., the coherence of the spontaneous fMRI signal between different brain regions. This potentially provides new possibilities to investigate sleep brain activity (for current methods see (Duyn, 2012)).

So far, only one study used EEG-fMRI to investigate brain network connectivity during sleep in typically developing children (Manning et al., 2013). In children with CSWS identified networks have been suggested to reflect both spike initiation and propagation pathways (Siniatchkin et al., 2010). The deactivations in structures of the default mode network were in line with the concept of epileptiform activity disrupting normal brain function.

The vast majority of studies presented so far involve a correlational approach. To establish causality manipulations are needed. Thus, a promising, not yet established method

for future pediatric sleep research is the modulation of sleep by non-pharmacological manipulations.

Modulation of sleep

In adults several studies provided evidence for methods successfully enhancing slow waves (for a review see (Bellesi et al., 2014)). The use of transcranial oscillatory Direct Current Stimulation (toDCS) at 0.75Hz induced an increase in the slow oscillation EEG activity (<1 Hz) which was associated with enhanced declarative memory performance, suggesting a causal role for slow waves in memory consolidation (Marshall et al., 2006). A recent study applying this method in children with ADHD reported similar results (Prehn-Kristensen et al., 2014).

Another study recently showed that specifically timed acoustic stimuli during slow wave sleep also induce an increase in the slow oscillation EEG activity again associated with enhanced declarative memory performance (Ngo et al., 2013b). To our knowledge, only one study investigated the feasibility of acoustic stimulation during slow wave sleep in children. In contrast to previous findings in adults, the authors found no effects of acoustic stimulation on EEG activity when applying the same stimulation protocol that had been used for the adult study (Piantoni et al., 2013). They hypothesize this lack of sensitivity to be due to the higher arousal threshold in children and recommend to consider increased sound levels for future acoustic stimulation studies in children.

Conclusions and future perspectives

Table 4 provides an overview of the presented current methods in sleep medicine and sleep research. Limitations and possible fields of application are summarized.

Questionnaires and diaries are a time- and cost-effective method. Subjective parental reports provide information about their children's habitual sleep and sleep problems such as difficulty falling asleep. However, if parents are unable to reliably report or if a more accurate estimation of nocturnal wake times is needed, complementary information provided by actigraphy might be helpful. In children suspected of having periodic limb movement disorders (PLMD), sleep-related breathing disorders or narcolepsy the gold standard for diagnosis remains PSG.

In pediatric sleep research sleep EEG is a well established method allowing the analysis of sleep structure (sleep stages) and specific sleep characteristics like slow waves or

spindels. HdEEG additionally allows topographical analysis. fMRI-EEG and the modulation of sleep are not yet established methods. However, especially the modulation of sleep might be a very promising method for future research and clinical application.

Table4: Overview of the current methods in sleep medicine and sleep research, limitations and possible fields of application.

Method	Properties	Field of application
Questionnaires and diaries	– Based on parental reports (or self-reports) – Subjective measures – Diaries: valid estimates of daily sleep onset, sleep offset and sleep period, however, limited accuracy of reported nocturnal wake times (18) – Questionnaires with satisfactory psychometric properties: valid estimates of general sleep behavior characteristics over extended time periods (weeks or months) (8) – Time- and cost-effective data collection and analysis	– Sleep medicine: identify sleep problems, screen for sleep disorders – Sleep research: investigate sleep behavior in typically developing children and adolescents
Actigraphy	– Based on movement – Objective measure – High sensitivity (detection of sleep) but, low specificity (detection of wakefulness) (21) – Data collection over multiple days in the natural environment – Simple data analysis using device-specific software – Moderate costs	– Sleep medicine: complementary behavioral information about nocturnal wake times – Sleep research: investigate sleep-wake patterns in typically developing children and adolescents
Polysomnography (PSG)	– Based on electrical brain activity, eye movement, submental and leg muscle activity, respiration and cardiac activity – Objective measures – In-lab sleep recordings of single nights – Time intensive and demanding data analysis – Expensive equipment	– Sleep medicine: diagnosis of periodic limb movement disorder (PLMD), obstructive sleep apnea (OSA) and narcolepsy (PSG and multiple sleep latency test) – Sleep research: investigate sleep characteristics in typically developing children and adolescents and in clinical populations
Electroencephalography (EEG)	– Based on electrical brain activity, eye movement and submental muscle activity – Objective measures – In-lab sleep recordings (but also at home recordings possible, using simplified equipment) – Visual sleep scoring – Expensive equipment	– Sleep research: investigate sleep characteristics in typically developing children and adolescents and in clinical populations
High-density electroencephalography (hd-EEG)	– Based on electrical brain activity, eye movement and submental muscle activity – Visualization of brain activity over the scalp (topographical distribution) for individual nights – Objective measures – Advanced analysis techniques necessary – In-lab sleep recordings of single nights – Expensive equipment	– Sleep research: investigate sleep characteristics, with a focus on regional differences, in typically developing children and adolescents and in clinical populations

2.2. Developmental trajectories of EEG sleep slow wave activity as a marker for brain and motor skill development during adolescence

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Abstract:

Reliable markers for brain maturation are important for the identification of neural deviations that eventually predict the development of mental illnesses. Recent studies have proposed topographical slow wave activity (SWA, EEG power between 0.75 – 4.5 Hz) during NREM sleep as a mirror of normal cortical development. However, studies about the longitudinal stability as well as the relationship with behavioural skills are needed before SWA topography may be considered a reliable marker for brain development. Thus, we examined 6 subjects (9.4-13.1 years, 3 females) longitudinally (3 nights ~ 2.5 years apart) using night high-density EEG and a visuomotor learning task. We found a steady increase of SWA for a frontal derivation and a decrease in a central cluster of electrodes across all subjects. Interestingly, SWA topography was relatively stable within each subject despite the large changes in EEG power indicating individual trait-like characteristics during development. Furthermore, the changes in SWA in the central cluster were related to the development of specific visuomotor skills. Thus, our findings support that SWA in the sleep EEG may be a reliable marker for (motor) skill and cortical development during childhood.

Introduction:

The time period between childhood and adolescence is associated with pronounced neurobiological and behavioural changes of the individual (Paus, 2005). In fact, this developmental phase has increasingly gained research attention because mental disorders often manifest during this period (e.g., attention deficit hyperactivity disorder, depression, schizophrenia and other early onset psychiatric disorders (Gogtay et al., 2004; Kessler et al., 2012)). Thus, establishing markers for healthy brain maturation is of significant relevance for the identification of neural deviations that may predict later illnesses. Those markers should have the following requirements: 1) a longitudinal time-course that parallels brain development, 2) sufficient stability (trait) across time within individuals and 3) a relationship with skill maturation (e.g., motor skills).

Evidence has increased that the sleep electroencephalogram (EEG) represents a "window into the developing brain" (Colrain and Baker, 2011b, a; Tarokh et al., 2011b). Especially, EEG sleep slow wave activity (SWA, EEG power between 0.75-4.5) parallels the trajectory of cortical development ((Ringli and Huber, 2011)). SWA is a major electrophysiological oscillation of non-rapid eye movement (NREM) sleep and a well-established marker of sleep homeostasis and sleep depth as underlined by a large body of work (Borbely and Achermann, 2000). For instance, it was noticed that SWA is strongly influenced by the duration of wakefulness preceding sleep by means of a SWA increase after sleep deprivation (Borbely and Achermann, 2000). EEG slow waves are related to the low-frequency oscillations (1 Hz) of the membrane potential of cortical neurons (Steriade et al., 1993a; Contreras and Steriade, 1995; Amzica and Steriade, 1998). The synchronized occurrence of slow oscillations across many cortical neurons lead to the characteristic slow waves measured in the surface EEG. SWA likely mirrors synaptic density because more and stronger synapses facilitate synchronization (Esser et al., 2007; Vyazovskiy et al., 2009). SWA profoundly changes throughout the first two decades of life, presumably reflecting the restructuring of synapses (i.e., number and efficiency, reviewed in (Ringli and Huber, 2011)). Indeed, longitudinal and cross-sectional studies in humans show that SWA follows the time course of an inverted U-shape with a maximum shortly before puberty, similarly to the time course of synaptic density (Feinberg, 1982; Zuo et al., 2005; Campbell and Feinberg, 2009; Feinberg and Campbell, 2010; Feinberg et al., 2011b). A recent study including subjects between 4 and 19 years of age supports the association between EEG derived SWA and grey matter volume assessed by MRI in different cortical regions (Buchmann et al., 2011). Other

studies demonstrated that the development of SWA shows regional differences (Jenni et al., 2005a; Kurth et al., 2010a; Feinberg et al., 2011a) and so does the thinning of the cortex measured in cross-sectional and longitudinal MRI studies (Gogtay et al., 2004; Shaw et al., 2008). Cortical restructuring during adolescence is characterized by a pruning of synapses. Interestingly, this pruning process is not equal across the cortex (i.e., starts in primary cortices, moves anterior and outward and ends in the prefrontal cortex (Gogtay et al., 2004; Shaw et al., 2008)). In a longitudinal study of sleep EEG recordings during development, Feinberg et al. (Feinberg et al., 2011a) reported that the SWA decline shows regional differences and follows a posterior (occipital electrode) to anterior (frontal electrode) maturational pattern. Their topographical analysis, however, was limited to 5 referential electrodes. Using high-density EEG (hdEEG, 128 electrodes) in a cross-sectional design, Kurth et al. (Kurth et al., 2010a) demonstrated that the region expressing maximal SWA relative to other brain regions shifts from posterior to anterior regions in 2-25 years old subjects. More specifically, maximal SWA in frontal derivations is reached during adolescence and therefore seems to parallel cortical brain development. However, longitudinal studies are needed to test this idea. In fact, a longitudinal setting would allow detecting changes in SWA at both the group and the individual level.

During adulthood SWA shows a prefrontal maximum that varies profoundly between subjects, while within subject topographies are relatively stable across repeated sleep measurements (and thus are considered trait-like (Finelli et al., 2001b; Finelli et al., 2001a)). The question arises whether such trait-like features of SWA are also observed during adolescence despite the pronounced decrease of SWA (i.e., a decrease by about 60% between 11 and 16 years (Feinberg et al., 2006; Campbell and Feinberg, 2009))? Tarokh et al. (Tarokh et al., 2011a) provides first evidence that trait-like aspects in the sleep EEG power spectra persist across adolescence despite considerable cortical restructuring. Our study further aimed to characterize the trait-like topographical aspects of SWA that remain stable across adolescence and to differentiate them from aspects that change within and between subjects across this time period.

Finally, there is increasing evidence that SWA is a reliable indicator of synapse number and efficiency. Synaptic remodelling is the basis of learning and memory, and therefore essential for the development of specific functions (e.g., motor skills). Thus, from this point of view SWA changes should be related to or even involved in the maturation of specific skills. In fact, Kurth et al. (Kurth et al., 2012) found that the maturity of slow wave topography was related to complex motor task performance. Furthermore, correlations

between motor performance of visuomotor learning and SWA activity in an adult population were reported (Huber et al., 2004). Thus, here we investigated how individual changes in SWA are related to behavioural changes in a visuomotor task across adolescence.

Methods:

Subjects

6 subjects (between 9.4 and 13.1 years, 3 females) from a larger dataset published in Kurth et al. (Kurth et al., 2010a) participated in the study. All of them underwent a telephone and questionnaire screening to exclude subjects with sleep disorders, chronic diseases, personal or familial history of psychopathology, current use of psychoactive agents or medications and left-handedness. Furthermore, none of the subjects travelled across more than one time-zone in the 4 months prior to the study. Study procedures were approved by the Cantonal Ethics Committee in Zurich, and the study was performed according to the declaration of Helsinki. Subjects of full age gave written informed consent after being explained the study procedures and aims in detail. Underage subjects gave oral and parents written informed consent to participate in the study.

To ensure stable conditions across all experimental nights, subjects were required to maintain a regular sleep schedule during one week prior to measurements. Actigraphy and sleep diaries (kept by participants or parents) were used to verify schedule compliance. Napping, caffeine consumption, medication, and alcohol consumption were not allowed 24-h prior to the sleep recordings. Since sleep rhythms vary across the menstrual cycle and show lowest variability in the follicular phase (Driver et al., 1996), we measured post-pubertal female subjects during this phase.

Procedure

All subjects had 3 experimental nights (baseline, follow-up night 1 [FU1], follow-up night 2 [FU2]) with 2.6 ± 0.5 years and 2.5 ± 0.1 years in between, respectively. The procedure was exactly the same for the 3 conditions including a visuomotor learning task performed in the evening and all-night sleep hdEEG recordings. Subjects went to bed according to their preferred bedtime and subjects were awakened in the morning according to educational or vocational obligations, which resulted in variable sleep durations.

Sleep EEG recordings and analysis

HdEEG nets (Electrical Geodesics Inc.) with 128 electrodes (including EOG) were adjusted to the vertex (Cz) and mastoids and subsequently filled with gel electrolyte. In addition, two submental EMG electrodes were applied. Besides high temporal resolution, the hdEEG provides good spatial resolution allowing the analysis of topographical and local aspects of sleep (Lustenberger and Huber, 2012). The signals were referenced to Cz and digitized at 500 Hz (band-pass filtering 0.01-200 Hz). Offline the signal was high-pass filtered at 0.5 Hz, low-pass filtered at 50 Hz and downsampled to 128 Hz. Sleep data was visually scored in 20 s epochs according to the American Academy of Sleep Medicine standard criteria (Iber et al., 2007). Artifact removal was done visually and if power exceeded a threshold based on a sliding mean of power in the 0.75-4.5 Hz or 20-30 Hz range (Huber et al., 2000).

EEG spectral power of consecutive 20-s epoch was calculated using a Fast Fourier Routine (Hanning window, average of five 4 s windows, frequency resolution 0.25 Hz). The spectral analysis was performed for the first hour of artifact-free NREM sleep. We selected this interval because (1) it was used by Kurth et al. (Kurth et al., 2010a) to map SWA topography in the first two decades of life in a cross-sectional design, (2) it belongs to the most consolidated part of sleep, (3) it includes the same number of epochs for all subjects, and (4) some subjects had several bad quality EEG electrode recordings towards the end of the sleep period. Spectral analysis was performed for all marginal electrodes (109), which allowed a topographical mapping of the data. EEG data was average referenced only including good quality channels. Bad quality channels (on average 2.4 ± 2.3 per night) were interpolated using a spherical interpolation provided by EEGLAB toolbox (Delorme and Makeig, 2004). EEG power values for each electrode within a map were normalized by dividing power at each electrode by average power across all electrodes (Kurth et al., 2012). We used this normalization, because we were interested in regional changes rather than the gross changes in signal amplitude that occur in all derivations during adolescence (see introduction).

Sleep EEG frequency bands were defined according to Kurth et al. (Kurth et al., 2010a). We focused on SWA (1-4.5 Hz), since power in this frequency range was shown to exhibit prominent age dependent changes (Feinberg et al., 2006; Kurth et al., 2010a; Feinberg et al., 2011a) and has been related to behaviour (Huber et al., 2004; Kurth et al., 2012).

In a first analysis to assess trajectories across age, we counted the number of subjects that showed consistent changes in SWA across the 3 measurements. More specifically, we identified electrodes in each subject that showed a steady increase or decrease in SWA by

means of an increase across all 3 measurements or a decrease, respectively. We then identified electrodes with a steady increase/decrease that were found in the vast majority of subjects, i.e. at least in 5 of 6 subjects. Regions of interest (ROI) were defined as the biggest clusters of electrodes (maximal number of adjacent electrodes/electrode) that followed a steady increase or decrease of SWA across the 3 time points in at least 5 of 6 subjects.

To quantify the degree of similarity of topographical maps within individuals compared to between individuals we calculated the Manhattan distance (Finelli et al., 2001b). To do so, we calculated the sum of absolute difference at all 109 derivations (excluding marginal electrodes, normalized power values) for all possible combinations within and between subjects (for details see (Finelli et al., 2001b)).

Visuomotor task

In the evening a visuomotor target reaching task was performed using the Motor Task Manager (ETT, Genova, Italy). Subjects moved a cursor with their right hand on a digitizing tablet from a central starting position to one of four targets displayed on a screen. Targets were separated by 45° and distance was kept by 8 cm to the common starting point. Targets were presented every second in a pseudo-randomized order. Subjects were instructed to reach to the highlighted target and go back to the starting point within this second in one straightforward movement as precise and smooth as possible. They first performed two blocks (including 44 movements each) to familiarize with the testing device and the movement. The third block was selected for the analysis. We focussed on two specific variables that were used in prior studies in a slightly different form and task design, and have been related to SWA changes (Huber et al., 2004; Huber et al., 2006): 1) Mean Absolute Directional Error, which measures the angular deviation from the ideal trajectory at the point of peak movement velocity; 2) Absolute Normalized Movement Area that represents the area enclosed by the hand-path divided by the squared path length. We computed the standard deviation of this area between the movements within the block (variability, a measure that might be closely related to inter-joint coordination (Krakauer et al., 1999; Huber et al., 2006)). Movement errors (i.e., when subjects moved towards the wrong target or when the trajectory was not executed in one movement) and outliers (Directional Error > 2.5*SD of the average across the block) were excluded.

Statistical analysis

We used R statistical software for all statistical analyses. We first pooled the data (EEG and behaviour) of the three measurements (3 observations per subject, 18 observations in total) to relate normalized SWA of the selected ROIs to the motor task variables. We further controlled for age, number of excluded trials and movement onset time in a partial correlation design using Pearson correlations. We repeated the same analysis with using the differences between baseline measurement and FU1, and between FU1 and FU2, resulting in 12 observations (2 differences per subject). To exclude the possibility that repeated observations per subject drive significant correlations, we performed a linear model of the residuals and included subjects as a random factor to test whether repeated observations significantly contribute to the model and affected our results. The factor "subjects" had no significant effect on the models and our results, and we therefore used the p-value provided from the linear model that did not included subject as a random factor. P-values < 0.05 were considered significant.

Results:

Intra- and interindividual changes, and individual stability of SWA topography

The topography of SWA for each subject and night is plotted in Figure 1. SWA over frontal areas increases (frontalization) and decreases over central areas. A more quantitative analysis of this effect shows that for a prefrontal electrode SWA in all subjects steadily increases across the 3 time points (Figure 2). In contrast, a specific central cluster of electrodes shows a constant decrease in SWA across all subjects.

Besides substantial SWA changes across development, characteristics of the SWA topography are also stable within-subjects even though years are between the measurements (Figure 1). In addition, these stable aspects of SWA topography vary substantially across individuals. For example, subject #3 has a clear right compared to left side predominance of SWA over the frontal cortex across all measurements, whereas subject #2 shows a central predominance compared to other regions (Figure 1). Thus, we see some stability (trait) of SWA topography across adolescence even in the face of pronounced developmental changes.

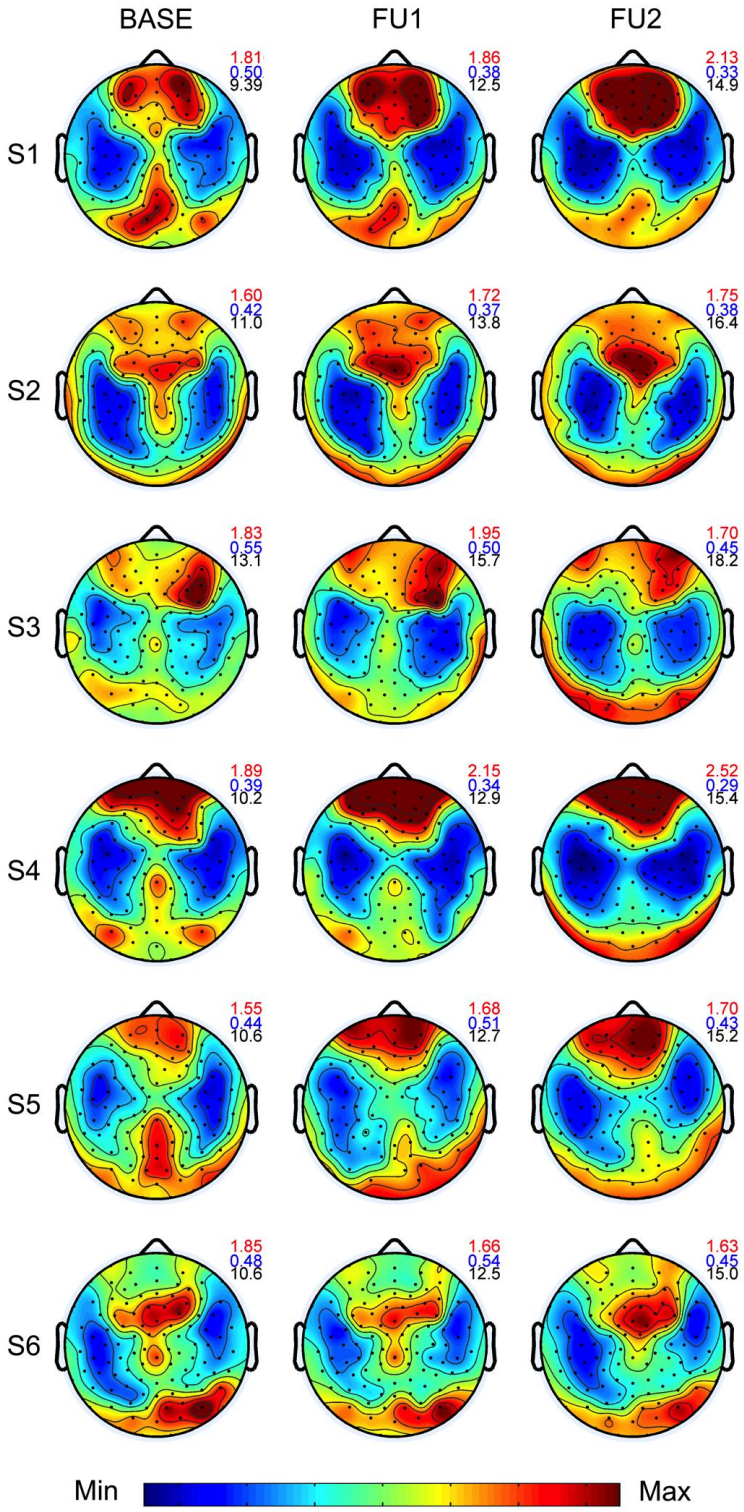

Figure 1: Topographical distribution of normalized SWA (EEG power 1-4.5Hz) of the first hour of NREM sleep for 6 subjects (rows) and 3 nights (column) each. On the topographical plot, hot colors denote maximal relative SWA and cold colors minimal activity. Numbers in the top right corner of the maps indicate the maximal (red) and the minimal (blue) normalized SWA, and the age of the subject in years (black). BASE: Baseline.

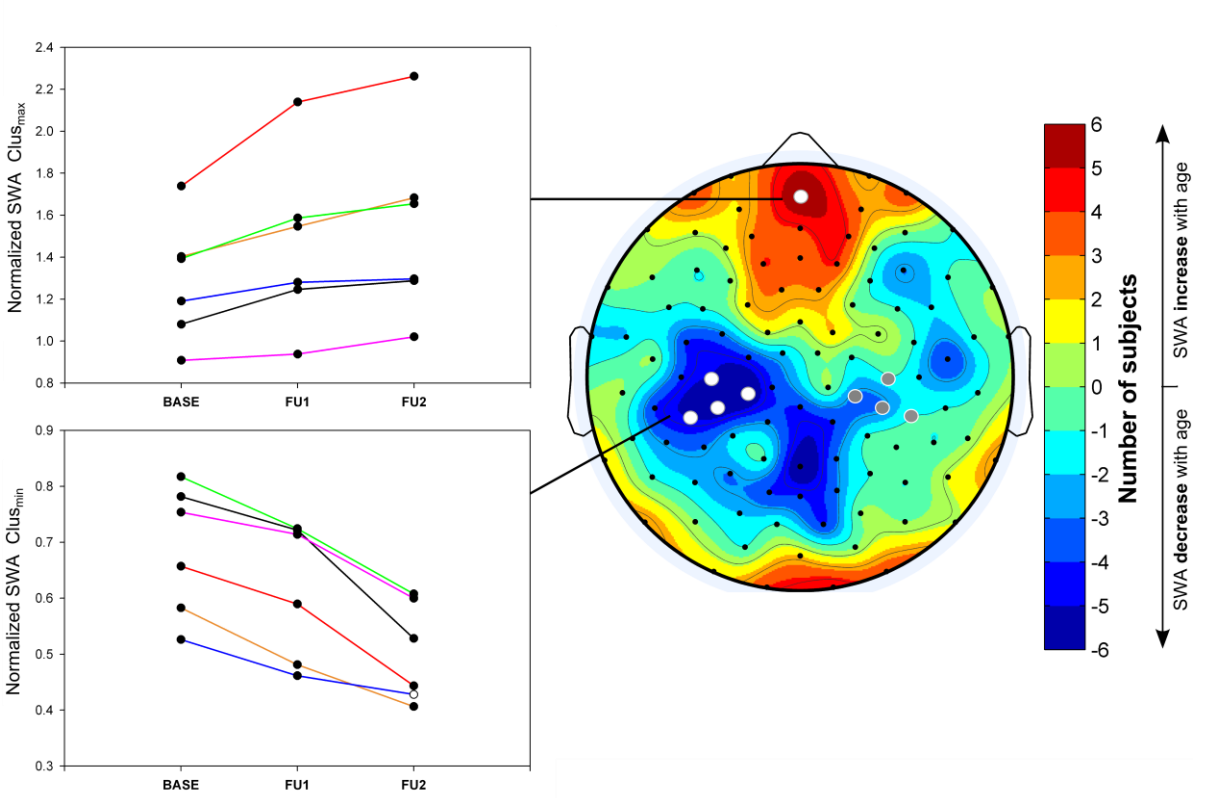

Figure 2: Individual SWA trajectories for two specific regions of interest (ROI, white circles) and all 3 time points (Base, FU1, FU2). The maximal number of electrodes (white marked) in a regional cluster with a steady increase (frontal electrode) or decrease (central cluster) in at least 5 of 6 subjects (topographical plot) was defined as a ROI. Subjects in the trajectory plots are color-coded (also used in Figure 4). A control region mirror-inverted to the central cluster is highlighted with grey marked electrodes (depicted for further correlational analyses, see results). SWA in this control region shows no consistent decrease across subjects. BASE: Baseline measurement, mean age 10.8 years; FU1: 1st follow-up measurement, mean age 13.4 years; FU2: 2nd follow-up measurement, mean age 15.9 years.

We further quantified this observation by calculating the Manhattan distance that estimates the correspondence across topographical maps. We found that correspondence or similarity across SWA topographies was significantly higher (lower Manhattan distance) for within subject comparisons (on average 12.05 ± 0.59 s.e.m.) than between subject comparisons (on average 19.38 ± 0.31 s.e.m.; Figure 3). In particular, all 6 subject had significantly less distance within their topographies (Baseline-FU1 and FU1-FU2) compared to all possible combinations of their distances to other subjects' topographical maps (all $p<0.05$).

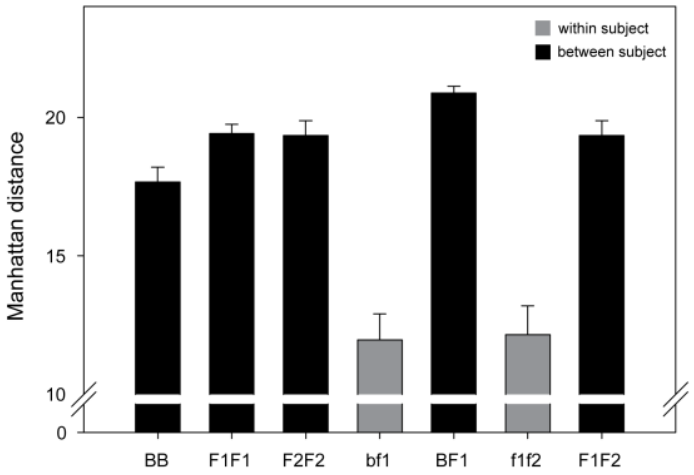

Figure 3: Error bars (mean + s.e.m.) of averaged Manhattan distances between individual topographical maps for SWA. Comparison of all pairs of topographies in baseline sleep (BB), FU1 (F1F1), FU2 (F2F2), baseline to FU1 (BF1), and FU1 to FU2 (F1F2) of different individuals to maps of baseline to FU1 (bf1) and FU1 to FU2 (f1f2) within the same individual. Lower distances represent more similar topographies. The intra-individual distances of bf1 and f1f2 were significantly different to all inter-individual distances (BB, F1F1, F2F2, BF1, F1F2; all $p<0.05$).

SWA changes and visuomotor skills

We further investigated whether SWA and SWA changes across the measurements in our selected ROIs are related to behavioural measures (Directional Error and variability of the Normalized Movement Area) of a visuomotor reaching task. Figure 4 further illustrates the individual trajectories of the Directional Error and the variability of the Normalized Movement Area.

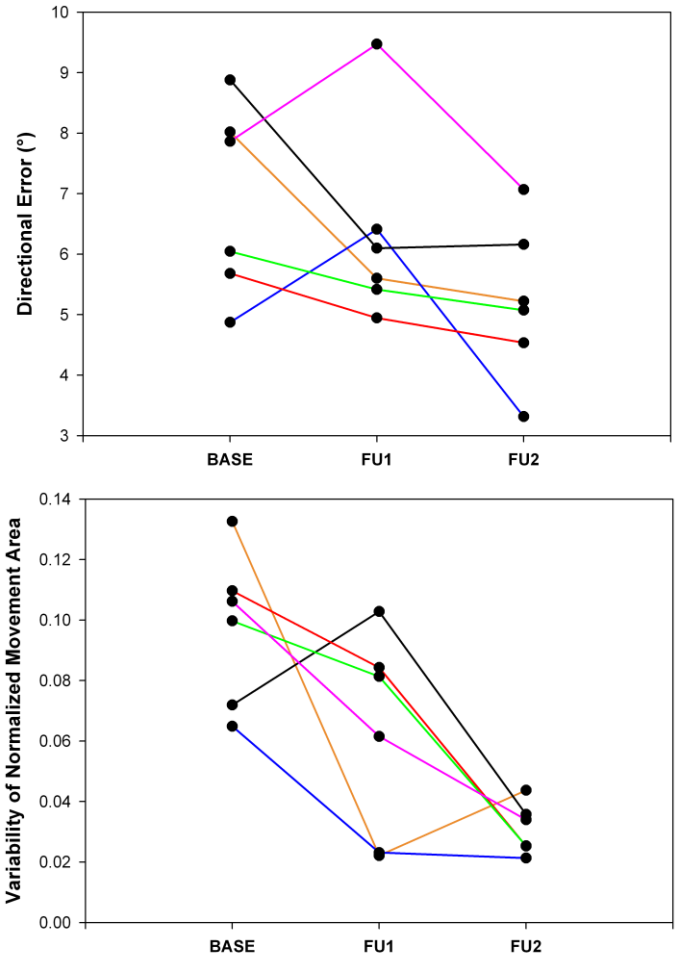

Figure 4: Individual visuomotor performance trajectories in the course of the 3 measurements. Upper panel illustrates Directional Error and lower panel variability (standard deviation) of the Normalized Movement Area. Subjects are colour coded (q.v. Figure 2).

We found a significant positive correlation between SWA in the central ROI and the variability of the Normalized Movement Area ($r(18) = 0.58$, $p = 0.01$; Figure 5A). Moreover, also the differences between the measurements (baseline-FU1, and FU1-FU2) of these two variables, which reflect the developmental changes over time, were positively related ($r(12) = 0.67$, $p = 0.02$; Figure 5B). Variability of the Normalized Movement Area was not correlated with the frontal ROI ($r(18) = 0.02$, $p = 0.95$; $r(12) = 0.09$, $p = 0.78$). Mean Directional Error was negatively associated with the SWA of the frontal ROI ($r(18) = -0.49$, $p = 0.03$), but no significant relation was found for the differences ($r(12) = 0.34$, $p = 0.28$). Furthermore, no association was observed between Directional Error and SWA of the central cluster, neither for all measurements ($r(18) = 0.20$, $p = 0.44$) nor for the differences ($r(12) = -0.31$, $p = 0.33$).

Of note, these partial correlations (covariates: age, number of included trials and movement onset time) also persisted after controlling for repeated measures (see methods).

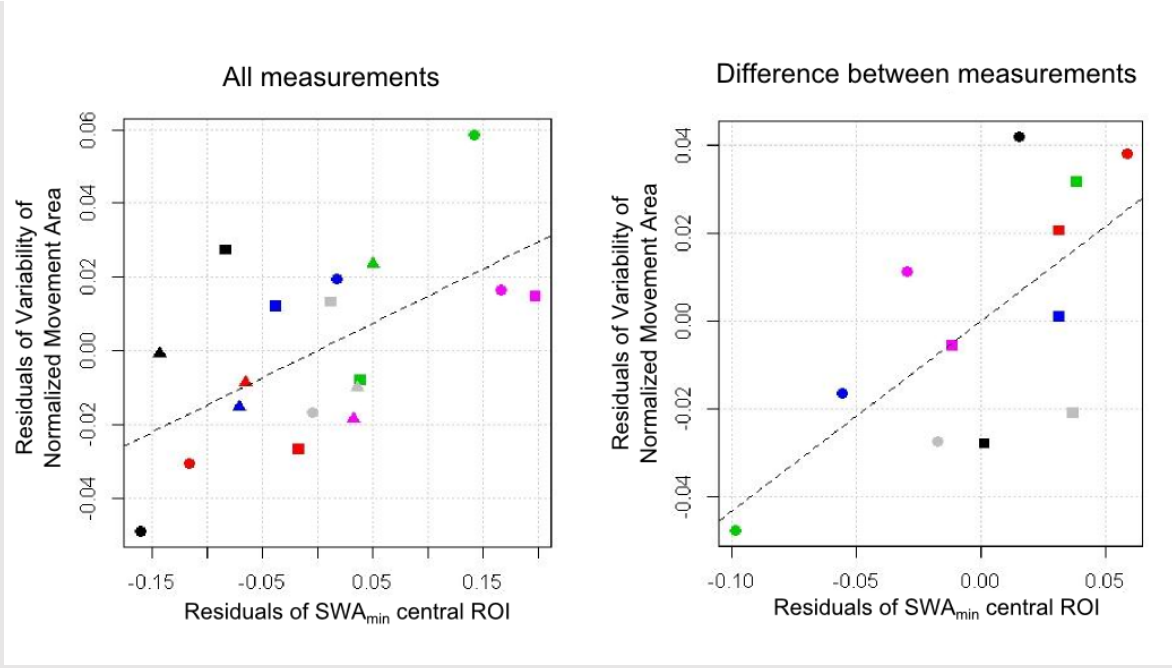

Figure 5: Scatter plot between residuals (partial correlation design, controlled for age, number of included trials and movement onset time) of SWA of the central ROI and residuals of the variability of Normalized Movement Area for **A** all measurements and **B** the differences between measurements. Same colours belong to the same individual. Correlations remain similar when controlling for repeated measures per subject (see methods).

To identify whether SWA of our selected central ROI specifically predicts variability of the Normalized Movement Area or whether central SWA in general relates to motor skills, we performed additional correlative analyses. To do so, we defined another cluster of central electrodes that was mirror-inverted across the cortical midline to our central ROI and did not show a consistent decrease in SWA in all subjects (q.v. Figure 1, grey circle). We found no significant correlation between SWA in this cluster and variability of the Normalized Movement Area, neither for all measurements ($r(18) = 0.00$, $p = 0.99$) nor for the differences between the measurements ($r(12) = -0.23$, $p = 0.47$).

Discussion:

SWA provides a functional correlate of cortical maturation and skill development, and undergoes prominent changes until it reaches mature stages during adulthood (Ringli and Huber, 2011). In this study, we investigated SWA topography to be a reliable marker of healthy brain maturation in a unique longitudinal study design. We found a steady increase of SWA for a frontal derivation and a decrease in a central cluster of electrodes across all subjects, although pronounced changes across the years the topography remained rather stable within each subject. The changes in SWA for a central cluster were further related to the development of visuomotor skills.

Intra- and inter-individual change, and intra-individual stability (trait) of SWA topography

The topographical distribution of SWA showed steady within-subject changes across development. Figure 1 shows that normalized SWA increases in frontal regions and decreases in central regions. This finding is consistent across subjects as revealed in our trajectory analysis illustrated in Figure 2. The increased frontalization with age has already been reported in a cross-sectional study design (Kurth et al., 2010a), which is now strongly supported by our longitudinal findings. A consistent decrease of SWA across all subjects was found in a left central electrode cluster. This cluster is located over Brodmann Areas (BA) 3, 4 and 6 (defined using MRI based anatomical electrode localization of n = 40 subjects, (Kurth et al., 2010a; Kurth et al., 2012)). Those BAs include the primary sensory and motor cortex, and premotor and supplementary motor areas and are therefore important for simple and complex motor skills (Kurth et al., 2012). The decline in SWA might highlight ongoing synaptic pruning in this region, which is related to a thinning of the cortex. A possible explanation why this central ROI is restricted to the left side might be that all subjects were right handed and are therefore more using their left hemisphere to perform arm motor tasks, possibly leading to a more pronounced or faster specialization of this region. Further studies are needed to compare these findings in a left-handed study population.

In addition to prominent changes in SWA topography across adolescence, we also found strikingly stable aspects of SWA topography, similar to reports in adults (Finelli et al., 2001b). This finding is intriguing, because (1) all subjects show a fingerprint-like topography but vary profoundly between each other, and (2) several years are between the measurements. Along this line, using a central and occipital EEG derivation Tarokh et al. (Tarokh et al., 2011a) reported that morphology of the sleep EEG spectrum is largely preserved between

repeated measures (2-3 years in between) in adolescent subjects with SWA showing intraclass correlation coefficients (ICC, measure of stability) as high as those observed in adults. We add an important dimension to these findings by showing that also regional aspects (SWA topography) are highly conserved across adolescence. How can these trait-like aspects be explained? The sleep EEG seems to be highly determined by the genetic background for which evidence is provided in twin studies (De Gennaro et al., 2008; Landolt, 2011). Intriguingly, based on these observations SWA topography may represent a good phenotype even in periods of pronounced cortical restructuring.

SWA changes and visuomotor target reaching behaviour

A key indicator for successful motor skill acquisition is the reduction of variability (Cohen and Sternad, 2009). Furthermore, development is accompanied by a reduction in intra-individual variability in behavioural performance for different type of tasks, including motor skills (MacDonald et al., 2006). In our sample we see a progressive decrease of the variability of the Normalized Movement Area during target reaching in most of our subjects. SWA in the central ROI were predictive for the variability in the Normalized Movement Area, with lower SWA values related to lower variability. Furthermore, the more pronounced the SWA decrease across adolescence, the more improvement (reduction) in variability of the Normalized Movement Area was found, a measure that presumably reflects inter-joint coordination (Huber et al., 2006). Reductions in grey matter which coincides with synaptic pruning during adolescence could increase neural efficiency and decrease noise in cognitive functioning, leading to a decrease in intra-individual variability during development (Sowell et al., 2003; Gogtay et al., 2004; MacDonald et al., 2006). We can therefore argue that lower SWA in this central ROI might be related to a more mature cortical region, more efficient and specialized synapses and therefore a more stable reaching movement. As mentioned earlier, this central cluster is located over sensorimotor areas and is therefore important for a variety of motor skills, including inter-joint coordination. This regional specificity is further underlined by the finding that SWA in the frontal ROI was not correlated with the variability of the Normalized Movement Area.

Besides a decrease in variability of reaching movements, we further found a progressive increase in accuracy of the reaching movements in 4 of 6 subjects reflected in a reduction of the Directional Error. We found a significant negative correlation between relative SWA only in the frontal ROI with Directional Error, but not for the difference between the measurements. Furthermore, no correlation was found between the variability of

the Normalized Movement Area and SWA in the frontal ROI. Thus, the progressive frontalization (increase over time) is not related to the assessed motor skills. Better behavioural measures that are more likely related to the maturation of the frontal ROI are cognitive skills (e.g. executive functions) that are mainly controlled by the frontal cortex (Stuss, 2011).

Limitations and Outlook

Our results should be considered in the context of the small number of subjects included in our study which gives us low statistical power. However, several findings are consistent across all subjects and, thus, seem to be quite robust. A further limitation is that we do not have the data to relate the time dependent changes in SWA to longitudinal direct measures of cortical maturation (e.g., grey matter volume). However, during the baseline measurement we also obtained structural T1 MRI images and calculated grey matter volumes for specific regions using Freesurfer version stable v4.5.0 for Mac OS 10.5.2 (http://surfer.nmr.mgh.harvard.edu; see also (Dale et al., 1999; Fischl et al., 1999b)) as reported previously in (Buchmann et al., 2011). Since we have only 6 subjects (and only 1 observation each), statistical analysis is weak. Nevertheless, we found a significant positive correlation between the mean volume of the precentral, postcentral and middle frontal gyrus (located beneath our EEG defined central ROI) and SWA in this region (Pearson correlation, $r = 0.87$, $p = 0.025$, $n = 6$, age was not significantly correlated with SWA or cortical volume and therefore not included as a covariate). This finding provides a first indication that SWA of the central ROI is directly related to cortical volume. No correlation between the SWA of the frontal ROI and frontal cortex volume was found, however due to low number of subjects and only one electrode consistently contributing to the ROI this finding should be weighted accordingly. Future studies are needed that simultaneously record sleep EEG changes and MRI measures across adolescence in a longitudinal setting.

Conclusion

Our data show that SWA topography is a trait but also provides coherent developmental changes in central and frontal brain areas that can be tracked within and across individuals. Furthermore, our results show that the development of SWA is related to the development of visuomotor skills. In summary, our findings further support that SWA in the sleep EEG can be used as a marker for (motor) skill development and cortical restructuring during

adolescence. Thus, our findings should next be investigated in the context of clinical populations. In particular, individuals with attention-deficit hyperactivity disorder (ADHD) might be a population of interest, as they show a significant increase of SWA compared to controls in a central cluster of electrodes that overlap with our specific central ROI (Ringli et al., 2012).

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2.3. Sleep slow wave activity reveals developmental changes in experience-dependent plasticity

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Abstract

Experience-dependent plasticity, the ability of the brain to constantly adapt to an ever-changing environment, has been suggested to be highest during childhood and to decline thereafter. However, empirical evidence for this is rather scarce. Slow wave activity (SWA, EEG activity of 1 – 4.5 Hz) during deep sleep can be used as a marker of experience-dependent plasticity. For example, performing a visuomotor adaptation task in adults increased SWA during subsequent sleep over a locally restricted region of the right parietal cortex which is known to be involved in visuomotor adaptation. Here, we investigated whether local experience-dependent changes in SWA vary as a function of brain maturation. Three age-groups (children, adolescents and adults) participated in a high-density EEG study with two conditions ('baseline' and 'adaptation') of a visuomotor learning task. Compared to baseline condition, sleep SWA was increased after visuomotor adaptation in a cluster of eight electrodes over the right parietal cortex. The local boost in SWA was highest in children. Baseline SWA in the parietal cluster and right parietal MR gray matter volume which both indicate region-specific maturation were significantly correlated with the local increase in SWA. Our findings indicate that processes of brain maturation favour experience-dependent plasticity and determine how sensitive a specific brain region is for learning experiences. Moreover, our data confirm that SWA is a highly sensitive tool to map maturational differences in experience-dependent plasticity.

Introduction

Children spend much of their waking hours in school so as to acquire fundamental skills and knowledge about the world. This seems to make perfect sense, because the developing brain is assumed to be very sensitive to novel experiences, resulting in a high capability for learning and memory. However, little empirical evidence is actually available in humans to indicate that experiences have more profound and more long-lasting effects in early than in later life. Studies focusing on sensitive periods may give first hints on the modulating effect of age on experience-dependent plasticity. A sensitive period is a time window during development in which specific experiences have most profound effects on the brain and behaviour (Knudsen, 2004). First evidence for the existence of sensitive periods in humans comes from studies on language development and musical training. In these experiments, better performance and greater neuronal changes were found in adults who started to exercise these cognitive abilities early in life than those who started later on (Kuhl, 2010; Steele et al., 2013). However, the neurophysiological mechanisms of sensitive periods in humans remain largely unknown. One open issue in this context is whether local processes of cortical maturation are directly linked to the sensitivity of a region to respond to specific learning experiences, as has been suggested elsewhere (Penhune, 2011).

Importantly, experience-dependent plasticity is not restricted to the brain in the waking state but extends to the sleeping brain. An increase in synaptic density and efficacy has been shown to enhance neuronal synchronization, which in turn results in increased sleep slow wave activity (SWA; EEG activity at a frequency of 1 – 4.5 Hz which hallmarks deep slow-wave sleep; (Esser et al., 2007; Dash et al., 2009; Vyazovskiy et al., 2009)). Specific learning experiences involving, for instance, intense visuomotor adaptation during the day resulted in an increase in post-learning SWA specifically over the cortical region that was shown to be involved during learning (Huber et al., 2004; Hanlon et al., 2009; Landsness et al., 2009; Määttä et al., 2010; Mascetti et al., 2013). Brain maturation is another factor that strongly affects sleep SWA. More specifically, not only has the SWA peak been found to parallel the posterior-to-anterior neurodevelopmental trajectory that has been described for grey matter volume, an anatomical marker of cortical maturation (Sowell et al., 2003; Casey et al., 2005), but SWA was also significantly correlated with grey matter volume (Kurth et al., 2010; Buchmann et al., 2011). In the present study, we investigated in children (9-11yrs, N = 15), adolescents (12-17yrs, N = 14) and adults (18-27yrs; N = 17) i) the effects of visuomotor adaptation on local SWA during the post-learning night and ii) the association between

measures of cortical maturation (i.e. grey matter volume and baseline SWA) and the local increase in SWA after visuomotor adaptation.

Methods

Subjects

Fifteen children between 9–11 years (mean ± SEM: 10.27 ± 0.22 years; 6 females, 9 males), fourteen adolescents between 12–17 yrs (15.51 ± 0.36; 2 females, 12 males) and seventeen adults (21.25 ± 0.73 years; 3 females, 14 males) participated in the study. Structural MRI data was available in thirty-seven of these participants (children N = 13, adolescents N = 14, adults N = 10) because they had also participated in another experiment in which these measures were recorded (whole data set is published in (Buchmann et al., 2011)). The participants were recruited via advertisements placed at the university, at the children's hospital and in local newspapers. Interviews with the parents and children as well as standardized questionnaires ensured that the children had no behavioural problems, cognitive impairments or sleep disorders. Subjects had no history of any neurological or psychiatric disorder and did not take any medication at the time of the experiment. The study was approved by the local ethics committee, and subjects gave written informed consent before participating. For the children and adolescents this was accomplished by a parent. Additionally, all children and adolescents provided verbal assent.

Procedure

Each subject participated in two conditions ('baseline' and 'adaptation') according to a within-subject cross-over design, with the order of conditions balanced across subjects. The two conditions for each participant were separated by at least one week. In both experimental conditions subjects came to the sleep lab around 3 hrs before subjects' habitual bedtime. The high-density electrodes net was placed immediately thereafter. In each condition subjects performed on a visuomotor task in the evening and went to bed thereafter.

Visuomotor Adaptation Task

In order to investigate local changes in sleep SWA we chose the visuomotor adaptation task because it is well-known to involve a specific region over the right parietal cortex (Ghilardi

et al., 2000). This task requires the subjects to move a cursor from a starting point to one of four possible targets thereby receiving a visual feedback about their hand movements on the screen in front of them. The experimenter permanently stood next to the subject during task performance to intervene in the case of a performance decrease due to lack of motivation or changes in posture (as a possible strategy to deal with the imposed rotation). In comparison to previous experiments in adults (Huber et al., 2004; Landsness et al., 2009; Määttä et al., 2010), we decided to use an easier version of the visuomotor adaptation task with only four instead of eight possible targets in order to make this task feasible for children. This was based on 1) previous findings demonstrating that implicit motor learning was lower in children as compared to adults (Fletcher et al., 2000; Thomas et al., 2004; Wilhelm et al., 2008), 2) on our own pilot data showing a high rate of dropouts in the learning session when using the 8-targets task version, and 3) the fact that a 4-target version of the task has been successfully used in almost all of the previous studies in children (e.g. (Ferrel et al., 2001; Contreras-Vidal et al., 2005)). With the reduction to four targets, we intended to reduce the length of the test period (to avoid an increase in tiredness and a decrease in motivation at the end of the blocks) as well as the difficulty of the task. In one of two conditions (i.e. the adaptation condition) the feedback was rotated at a fixed angle. Subjects performed on a total of seven blocks each including three trials of the same rotation angle with all of them being separated by a short break. In each of the trials, subjects had to execute 44 movements to one of the four targets in a pseudo-randomized order. To familiarize the subjects to the requirements of the task, the learning session started with one block without any feedback rotation (block 1; B1). Thereafter, feedback was rotated at a block-wise increasing angle (B2 - 15°, B3 - 30 °, B4 - 45°, B5 - 60°). Actual visuomotor performance was tested in a separate test block at the end of the test session (B7). Preceding this test block a block of 0° rotation (B6) was introduced aiming at washing out any residual rotation of the internal model. We computed the directional error for each movement as the angle between the line from the initial hand position to the position of the target and the line to the position of the hand at the peak outward velocity (DEPV). As a measure of the final performance level in the adaptation condition we calculated the average performance across all 44 movements in the last trial of block 7. In the baseline condition, subjects had to execute the same amount of hand movements from the starting point to one of four targets as in the adaptation condition but feedback was not rotated at any time. We chose exactly this kind of task to reduce the possibility that the three age-groups can use different strategies. Previous experiments have uncovered two possible strategies that can be used in such a visuomotor

adaptation task, i.e. explicit and implicit strategies (Benson et al., 2011; Taylor and Ivry, 2012; Taylor et al., 2014). However, the spontaneous generation of explicit knowledge in a visuomotor adaptation task - without any additional task instructions given by the experimenter - depends on specific features that were not present in our task. These conditions are i) sufficient amount of rest between trials which allows for movement corrections and the conscious reflection of possible sources of performance errors (in our study, targets were presented one after another at an interval of one second which forced the subjects to quickly execute the movements) and ii) the abrupt imposing of the final rotation which produces a large error signal and thereby creates a situation where subjects might become aware of the perturbation (the rotation was gradually imposed in our study). Importantly, even if a protocol fulfils these two requirements, the actual number of subjects that gain insight into the nature of the imposed perturbation and therefore are able to use an explicit strategy to improve their performance is extremely low ((Benson et al., 2011) found only 3 out of 27 adult subjects). Accordingly, it is very unlikely that the three age-groups differed with regard to the use of their strategy because with this version of the task it was nearly impossible to generate any explicit knowledge which would be the bases for using an explicit strategy.

EEG Recording, Preprocessing and Analysis

All-night sleep EEG, electrooculogram, and electromyogram were recorded in the sleep laboratory of the University Children's Hospital Zürich (Switzerland). All participants were monitored during both experimental nights using high-density sleep EEG (Electrical Geodesics Sensor Net for long-term monitoring, 128 channels, referenced to a vertex electrode for direct visualization and to the average across all channels for data analysis). Data were sampled at 500 Hz (0.01–200 Hz). Offline, the EEG was bandpass filtered (0.5–50 Hz) and downsampled to 128 Hz. Artifacts were rejected on a 20 s basis after visual inspection and if power exceeded a threshold based on a mean power value in the 0.75– 4.5 and 20–30 Hz bands (Huber et al., 2000). Poor quality EEG channels were excluded (mean number of excluded channel: 2.01 ± 0.22; range: 0-12) and for the topographical analyses, data of excluded channels were interpolated by the method of spherical linear interpolation. All further analyses were based on re-referenced data: for every EEG sample, the value of each channel was divided by the average value across all 109 channels above the ears. The EEG was visually scored for sleep stages at frontal, central and occipital electrodes (20 s epochs) based on American Academy of Sleep Medicine standard criteria (Iber et al., 2007).

For qualitative exploration, spectral analysis was performed for all channels [fast Fourier transform routine, Hanning window, 20 s epochs (averages of five 4-s epochs), frequency resolution of 0.25 Hz]. The 20 s spectral power values were then averaged for a certain time window. Subsequent analyses were restricted to the slow wave frequency band (SWA; 1–4.5 Hz). Because we focused on experience-dependent local SWA changes, SWA was normalized by dividing SWA at a single electrode by the average SWA over all 109 electrodes as done previously (e.g. (Huber et al., 2004)). Absolute SWA (i.e. without normalization) is subject to profound day-to-day changes which makes this measure less sensitive to uncover small experience-dependent local changes in SWA. Nevertheless, we also analysed experience-dependent changes in absolute SWA and found the same effects that were – as expected – less pronounced as compared to the normalized data. We defined as a cluster of electrodes at least seven significant neighbouring electrodes. We chose this number of electrodes because when calculating 109 statistical tests six electrodes will become significant by chance given a significance level of 5% (which means that the calculation of 100 statistical tests will lead to 5 significant results without any real difference between the conditions). Please note that this threshold is conservative because the likelihood of these six electrodes being neighbouring electrodes is much lower.

Structural MRI and Surface Based Analysis

All images were obtained on the same 3-T scanner, a General Electrics Signa HDx. We used a T1-weighted gradient-echo whole-brain image, repetition time 8.928 ms, echo time 3.496 ms, and flip angle 13°; image resolution in x--y--z direction was 256 x 256 x 140 voxels, resulting in a resolution of 0.938 x 0.938 x 1.2 mm.

Parietal gray matter volumes were calculated using Freesurfer version stable v4.5.0 for Mac OS 10.5.2 (http://surfer.nmr.mgh.harvard.edu; see also (Dale et al., 1999; Fischl et al., 1999a)). In this software, the T1 images are analyzed using the recon-all procedure, which treats subcortical structures and cortical hemispheres separately. The hemispheres are registered with a spherical atlas, which utilizes individual cortical folding patterns to match cortical geometry across subjects. This procedure allows parcellation of the cerebral cortex into gyral and sulcal units. It has been shown that Freesurfer statistics are robust against white noise in the images and that results are similar for multiple T1 images and one T1 image (Jovicich et al., 2009). Areas from the surface-based analysis were fused to form the parietal lobes left and right.

Statistical analyses

Statistical analysis of directional error at peak velocity was based on 4x3x3 analyses of variance (ANOVA) including the group factor ‘age-group’ (children, adolescents and adults) and the repeated measures factors ‘block’ representing the four blocks of gradual adaptation and the factor ‘trial’ (three trials of the same rotation within each block). Analyses of experience-dependent local changes in SWA were based on 2 x 3 ANOVA including the repeated measures factor ‘condition’ (baseline vs. adaptation) and the group factor ‘age-group’. In case of a significant main effect of age-group in these ANOVAs, post-hoc comparisons using Tukey HSD were calculated to further elucidate the effect of ‘age-group’. In case of a significant interaction including the factor ‘age-group’ separate post-hoc t-tests were calculated. Correlation analyses were conducted using Pearson's correlation coefficient. The level of significance was set to $P = 0.05$.

Results

Visuomotor performance before sleep

In the adaptation condition, subjects performed on seven blocks each including three trials of the same rotation angle with all blocks being separated by a short break. In each of the trials, subjects had to execute 44 movements to one of four targets. After one block without feedback rotation (B1), feedback was rotated at a block-wise increasing angle in the subsequent blocks (B2 - 15°, B3 - 30 °, B4 - 45°, B5 - 60°). All three age-groups were able to adapt to the imposed rotation as indicated by a decrease in the directional error at peak velocity (DEPV) from block 2 to block 5 as well as across the three trials of the same rotation in each block (main effect of 'block' $F(1.7, 68.1) = 135.85$, $p < 0.001$; main effect of 'trial': $F(1.8, 77.5) = 244.52$, $p < 0.001$; Figure 1C). In the final trial of block 7 (last 44 movements during the training session), adults outperformed children (mean DEPV ± SEM, adults: 12.76 ± 0.97; children; 18.6 ± 1.48; difference between both age-groups: $p = 0.026$) while no difference was found between children and adolescents (adolescents: 17.96 ± 2.21; $p > 0.95$)

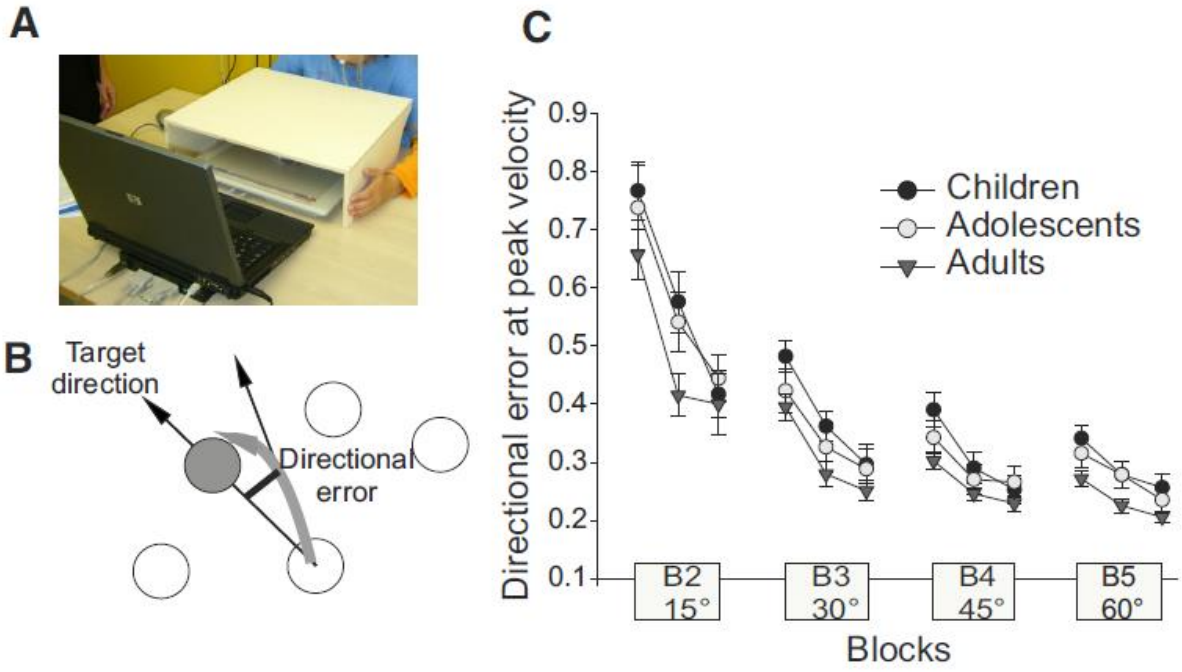

Figure 1. Visuomotor adaptation task and visuomotor performance in children, adolescents and adults. (A) During the visuomotor adaptation task, subjects sat in front of a screen which displayed a starting point in the middle of the screen and four possible target points. Subjects were required to move a cursor from a starting point to one of four targets while not seeing their movements because the hand was covered. Instead they received a feedback about their movement on the screen. (B) As a measure of each movement's quality, we computed the angle between the line from the initial hand position to the position of the target and the line to the position of the hand at the peak outward velocity (i.e., directional error at peak velocity, DEPV). (C) To measure the time course of visuomotor adaptation, the DEPV was normalized by dividing it to the actual rotation angle. All three age-groups were able to adapt to the imposed rotation as indicated by a decrease in DEPV within each block ($p < 0.001$ for main effect of 'trial').

Experience-dependent local changes in Slow Wave Activity

In both conditions, high-density EEG (128 electrodes) was recorded during the night. As expected, children, adolescents and adults differed significantly with respect to a number of sleep parameters (i.e. total sleep time, amount of sleep stage 2 and 3). Importantly, there were no differences in any sleep parameter when comparing 'baseline' with 'adaptation' condition (see Table 1 for sleep parameters and statistics).

Table1: Table 1. Sleep in both experimental conditions in all three age-groups

	Children		Adolescents		Adults		p–value
	Baseline	Adaptation	Baseline	Adaptation	Baseline	Adaptation	
Total Sleep Time (min)	524.2 ± 11.7	533.1 ± 8.7	449.1 ± 20.0	466.7 ± 14.4	436.7 ± 12.1	440.4 ± 9.4	0.017
Sleep latency (min)	26.0 ± 3.1	22.8 ± 3.4	15.9 ± 2.3	14.7 ± 2.5	21.3 ± 2.6	17.8 ± 2.0	0.02
Vigilance states in % of TST							
Wake	54.0 ± 10.2	34.3 ± 9.0	20.8 ± 4.8	34.7 ± 10.4	34.1 ± 8.4	24.3 ± 4.4	0.20
Stage 1	7.5 ± 0.8	6.7 ± 0.7	8.2 ± 1.4	7.1 ± 0.8	5.8 ± 0.5	6.3 ± 0.6	0.26
Stage 2	45.6 ± 2.1	49.1 ± 1.7	50.4 ± 1.4	51.6 ± 1.6	55.5 ± 1.7	54.4 ± 1.5	0.004
SWS	27.6 ± 2.2	24.6 ± 2.1	22.5 ± 1.5	21.6 ± 1.8	18.6 ± 2.2	19.6 ± 2.0	0.04
REM sleep	19.3 ± 1.0	19.5 ± 1.0	18.9 ± 1.5	19.6 ± 0.7	19.9 ± 1.2	19.8 ± 1.1	0.90

Mean (± s.e.m.) total sleep time (TST), sleep latency, i.e., the first occurrence of stage 2 sleep, determined with reference to the time of lights off and time spent awake, in stage 1 sleep, stage 2 sleep, slow wave sleep (SWS) and rapid eye movement (REM) sleep in percentage of total sleep time. The p-value for the main effect of 'age-group' in 2 ('condition') x 3 ('age-group') ANOVAs is shown in the last column (main effect 'condition' for all analyses $p > 0.12$).

To investigate whether visuomotor adaptation affects local SWA we calculated for each of the 128 electrodes a 2x3 ANOVA including the within subject-factor 'condition' (adaptation/baseline) and the between subject factor 'age-group' (children/adolescents/adults). During the post-learning night, SWA was increased in a cluster of eight electrodes that were located over the right parietal cortex (main effect of 'condition' for each of these electrodes $p < 0.05$; Figure 2A). To further investigate the modulating impact of age on experience-dependent changes in SWA, we averaged SWA over these eight electrodes and calculated a 2 ('condition') x 3 ('age-group') ANOVA. This analysis revealed that the impact of visuomotor adaptation on local SWA was critically modulated by the subjects' age (main effect of 'condition': $F(1,43) = 13.63$, $p < 0.001$; interaction 'condition' x 'age-group': $F(2,43) = 3.50$, $p = 0.039$). Post-hoc t-tests indicated that visuomotor adaptation strongly affects SWA in the parietal cluster in the group of children (adaptation vs. baseline: $p = 0.003$) but not in adolescents ($p = 0.20$) and adults ($p = 0.36$; Figure 2B). Accordingly, age was negatively correlated with the local increase in SWA ($r = -0.35$, $p = 0.018$; Figure 2C).

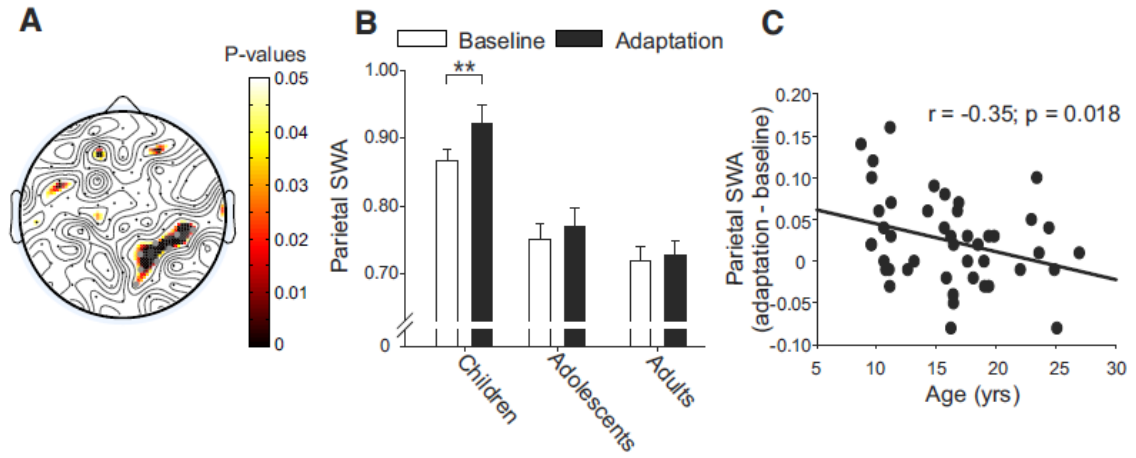

Figure2: Local changes in slow wave activity (SWA) after visuomotor adaptation and the modulating impact of age. (A) After visuomotor adaptation, SWA was increased in a cluster of eight electrodes located over the right parietal cortex (all electrodes marked as grey dots were $p < 0.05$ for main effect 'condition' (adaptation vs. baseline)). (B) Mean ± s.e.m. of SWA in the eight electrodes of the parietal cluster was significantly higher in the adaptation condition (black bars) compared to the baseline condition (white bars) in the group of children ($p = 0.003$) but not in adolescents and adults (both $p > 0.20$). (C) Age (in yrs) was negatively correlated with the increase in SWA (i.e. the difference in SWA between the adaptation and baseline condition) in the parietal cluster after visuomotor adaptation. ** $p < 0.01$

To analyse the time course of experience-dependent local changes in SWA across the night we calculated SWA in the parietal cluster in the first 30 minutes of NonREM sleep in each of the first five sleep cycles in both experimental conditions. In children, higher SWA in the parietal cluster was found after visuomotor adaptation as compared to baseline in the first four cycles (1^{st}, 2^{nd} and 4^{th} $p < 0.05$; 3rd $p < 0.10$; Figure 3A) whereas in adolescents and adults this was the case in two out of five cycles (adolescents 2^{nd} $p < 0.05$, 3^{rd} $p < 0.10$; adults 2^{nd} and 4^{th} $p < 0.05$; Figure 3B,C see also Figure 4 for the topoplots indicating local changes in SWA in the first four cycles in each of the three age-groups).

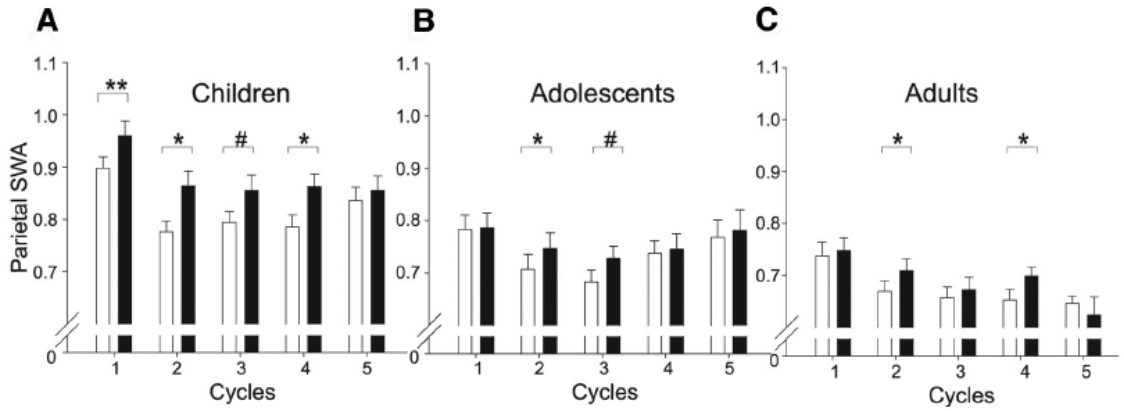

Figure 3. Time course of experience-dependent slow wave activity (SWA) changes across the night in all three age-groups. Mean ± s.e.m. of SWA in the eight electrodes of the parietal cluster in the first 30 minutes of NonRem sleep of the first five cycles in the adaptation (black bars) and the baseline condition (white bars) in (A) children, (B) adolescents and (C) adults. # $p < 0.10$; * $p < 0.05$; ** $p < 0.01$

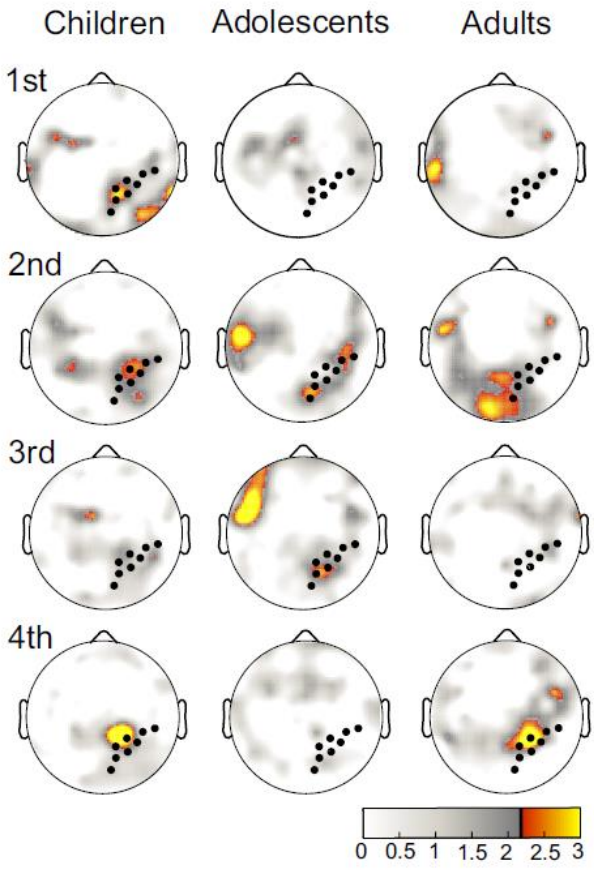

Figure 4. Local changes in SWA after visuomotor adaptation in all three age-groups for the first 30 minutes in each of the first four sleep cycles. T-values from 0 to 3 are presented for all electrodes. Regions of significantly increased SWA are shown from red to yellow (the critical T-value in all age-groups and cycles of 2.15 is marked by the black line in the colorbar). Note, that the right parietal cluster (as indicated by the black dots) is the one that most consistently showed a significant increase after visuomotor adaptation. The discussion of regions becoming significant in single cycles and age-groups (e.g. the frontocentral region in the 2nd and 3rd cycle in adolescents) is interesting but goes beyond the scope of this study.

In a next step, we tested whether the observed age-dependent differences in experience-dependent SWA changes are directly related to processes of cortical maturation. SWA is a valid marker of brain maturation as it was shown to be highest in regions undergoing cortical reorganization during maturation (Kurth et al., 2010a; Buchmann et al., 2011). We used SWA in the first 30 minutes of NonREM sleep in the baseline condition as a measure of cortical maturation. In line with previous imaging studies showing a posterior-to-anterior trajectory (Sowell et al., 2003; Casey et al., 2005), we found that the SWA peak shifted from occipital to frontal areas from childhood to adolescence (Figure 5A). Moreover, baseline SWA in the eight electrodes of interest (i.e. those electrodes over the parietal cortex that had been found to show increased SWA after visuomotor adaptation) was highest in the group of children (main effect of 'age-group': $F(2,42) = 17.87$, $p < 0.001$, children vs. adults $p < 0.001$; children vs. adolescents $p < 0.01$; adolescents vs. adults $p = 0.58$; Figure 5B). Importantly, baseline SWA in this cluster was significantly correlated with the increase in SWA after visuomotor adaptation in children, while in adults or adolescents such a correlation was not found (children $r = 0.70$, $p = 0.004$; adolescents $r = -0.06$, $p = 0.85$; adults $r = -0.26$, $p = 0.29$; Figure 5C). To verify that baseline SWA can indeed be used as a marker of maturation in the current study sample, we analyzed structural MRI markers of brain maturation in the region of interest (i.e. the right parietal lobe) in a subset of the study sample (N = 37, children N = 13, adolescents N = 14, adults N = 10). Grey matter volume in the right parietal lobe was significantly correlated with baseline SWA in the cluster of interest over the right parietal lobe ($r = 0.46$, $p = 0.004$; Figure 5D). Moreover, grey matter volume in the right parietal lobe correlated with the increase in SWA after visuomotor adaptation in the right parietal electrode cluster ($r = 0.46$, $p = 0.004$, age-corrected: $r = 0.35$, $p = 0.034$; Figure 5E). A lack of a correlation between grey matter volume in the left parietal lobe and the SWA increase in the right parietal cluster ($r = 0.27$, $p = 0.11$, age-corrected: $r = 0.11$, $p = 0.54$) points towards an effect which is specific for the region that is involved in the task rather than a general effect of brain maturation.

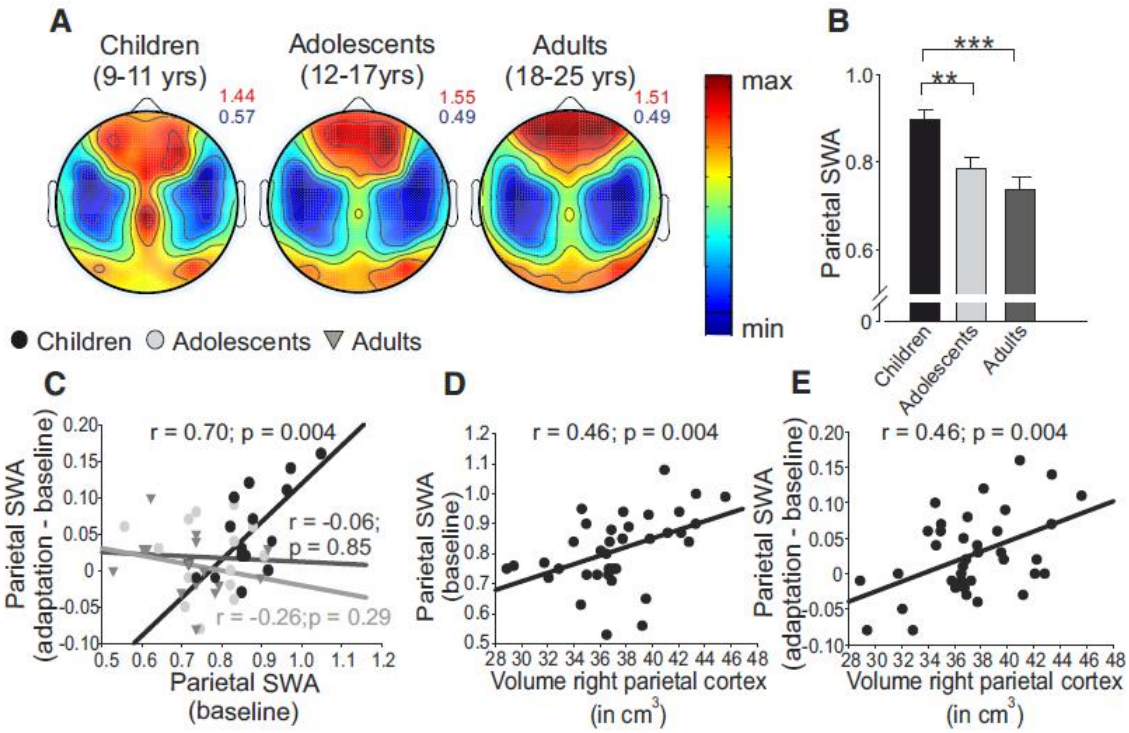

Figure 5. Slow wave activity (SWA) topography, baseline SWA in the parietal cluster and local increase in SWA after visuomotor adaptation. (A) Topography of SWA in the first 30 minutes of NonRem sleep in children, adolescents and adults in the baseline condition. (B) SWA in the parietal cluster recorded in the baseline condition was highest in children (** $p < 0.01$; *** $p < 0.001$) and (C) it was significantly correlated with the increase in SWA after visuomotor adaptation in children but not in adults and adolescents. (D) A significant correlation was found between grey matter volume in the right parietal lobe and baseline SWA in the cluster of interest over the right parietal lobe ($r = 0.46$, $p = 0.004$). (E) Grey matter volume in the right parietal lobe was correlated with the increase in SWA after visuomotor adaptation in the right parietal electrode cluster ($r = 0.46$, $p = 0.004$).

Discussion

We subjected children, adolescents and adults to a novel learning experience (i.e., a visuomotor learning task) that has previously been shown to activate circumscribed brain regions in a PET study (Brodmann Areas 40 and 7 which are located in the right parietal cortex; (Ghilardi et al., 2000)). In the post-learning night, we observed a gain in SWA in a cluster of eight electrodes over the right parietal scalp that was strongest in children compared to adolescents and adults. The finding of increased local SWA in a region that is known to be recruited during task performance is in line with a number of previous experiments in humans and rodents reporting a link between learning experiences during the wake phase and local SWA during subsequent sleep (Kattler et al., 1994; Huber et al., 2004; Huber et al., 2006; Hanlon et al., 2009; Mascetti et al., 2013). Slow wave activity in the surface EEG reflects a highly synchronous alternating pattern of neuronal firing (depolarization) and neuronal silence (hyperpolarization) in large neuronal networks (Steriade et al., 1993a). Novel learning can lead to the formation and selective elimination of synapses and the modulation of synaptic strength (for review see (Fu and Zuo, 2011)). An increase in synaptic density and efficacy has been shown to enhance neuronal synchronization which in turn results in greater SWA (Esser et al., 2007; Dash et al., 2009; Vyazovskiy et al., 2009). Thus, it is reasonable to assume that the local change in SWA in the right parietal cluster in the post-learning night reflects the increase in synaptic strength and/or density in this specific region induced by the learning task.

Our finding of greatest changes in local SWA in the group of children indicates that experience-dependent plasticity is highest during childhood and declines thereafter. These data is well in line with a number of previous findings pointing towards the existence of sensitive periods during human development for specific skills, that is second language-learning and musical training (Elbert et al., 1995; Schlaug et al., 1995; Nicholas and Geers, 2007; Bailey and Penhune, 2010; Kuhl, 2010; Penhune, 2011; Steele et al., 2013). In most of these experiments better performance was found in adults who started to train these cognitive abilities early in life when compared to those who started later on. As being discussed earlier (Penhune, 2011), these studies can only give indirect information on the modulating effect of age on experience-dependent plasticity as they are subject to multiple confounding factors. More specifically, one can hardly exclude that children (‘early learners’) and adults (‘late learners’) already differ with regard to pre-existing factors such as genetically determined differences in skill or individual differences in motivation and

training. These confounding factors were eliminated in our study by mapping local changes in SWA immediately after learning. Importantly, using this approach we were able to show that sensitive periods for visuomotor learning exist during human development.

The neurophysiogical mechanisms underlying sensitive periods in humans are not well understood. It has recently been argued that learning a specific skill induces profound and long-lasting effects in the brain and on behaviour at the time during which the involved cortical region undergoes maturation (Penhune, 2011; Steele et al., 2013). Maturation does not occur simultaneously in different brain regions but motor and sensory systems mature earliest, temporal and parietal association cortices mature next and prefrontal and lateral temporal regions mature latest (Sowell et al., 2003 adolescents and adults in our study may have shown lower experience-dependent plastic changes after visuomotor adaptation than children because their parietal cortex is already fully mature. Our findings on a strong correlation between grey matter volume in the right parietal cortex and the local experience-dependent increase in SWA over the right parietal scalp are in line with this notion. Moreover, SWA over the parietal cluster which can be used as an electrophysiological marker of this region's maturation {Buchmann, 2011, EEG sleep slow-wave activity as a mirror of cortical maturation; Gogtay et al., 2004; Casey et al., 2005; Kurth et al., 2010a; Kurth et al., 2012) was also significantly associated with the experience-dependent increase in SWA over this region. These findings suggest that i) actual processes of brain maturation within a specific region favour experience-dependent plasticity in this region and that ii) mapping local SWA during post-learning sleep is a sensitive tool to uncover this relationship. This suggestion is supported by an animal experiment studying the impact of sensory experiences on SWA in young and old mice and cats (Miyamoto et al., 2003). In this study, visual deprivation (i.e. dark rearing) of young mice and cats during the critical period for ocular dominance resulted in a dramatic reduction of SWA over the primary visual cortex whereas this effect was not observable in older animals. Future studies need to elucidate the exact time-courses of experience-dependent plasticity for a variety of cognitive tasks that depend on different brain regions. An estimation of the developmental time window where the brain is most vulnerable to the external input of specific experiences (i.e. language-learning or the acquisition of motor skills) can have important implications not only for educational policy, but also clinical therapy.

SWA is not only an indicator for experience-dependent plasticity but it is itself causally related to the long-term storage of newly acquired experiences. For example, task performance at a later recall was improved when SWA during post-learning sleep had been

boosted by auditory or electrical stimulation (Marshall et al., 2006; Ngo et al., 2013b). Thus, current models suggest a cycle of novel learning experiences leading to a local increase of SWA during sleep which in turn results in a strengthening and/or improvement of performance in the learned task after sleep (Nere et al., 2013; Rasch and Born, 2013). Whether visuomotor adaptation indeed develops optimally over time in children and whether this is related to their strong increase in local SWA after visuomotor adaptation needs to be studied in future experiments.

Moreover, SWA has recently been hypothesized to be actively engaged in processes of brain maturation (Campbell and Feinberg, 2009; Huber and Born, 2014). Brain maturation is a process of intense cortical reorganization characterized by a rapid increase in synaptic density, followed by an elimination of synapses (for a review see (Rakic et al., 1994)). In adolescent mice, the production of synapses was found to most efficiently occur during wakefulness whereas the elimination of synapses was most prominent during sleep (Maret et al., 2011). Here, we found that stimulating a specific brain region by a learning task at the developmental time of high synaptic density (as indicated by grey matter density and baseline SWA) results in a strong increase in SWA in the same region. This increase in SWA presumably reflects the learning-dependent increase in synaptic density/strength which may lead to a more efficient reorganization of the involved cortical circuits. Together with previous work revealing the importance of sleep for brain plasticity during development in animals (Frank et al., 2001; Jha et al., 2005), the current experiment indicates that studying processes during sleep may well be fruitful to improve our understanding of how experiences affect the brain during early development. Moreover, if indeed sleep is centrally involved in shaping our brain during development, disturbed or insufficient sleep during development might have negative consequences on cortical maturation.

A sensitive period within which an experience can induce major changes in the brain does not exclude that the same experience has no impact after closure of this window. Previous studies indeed reported an increase of SWA over the parietal cortex after visuomotor adaptation in a group of adults (Huber et al., 2004; Landsness et al., 2009). The weaker effect of visuomotor adaptation on SWA in our adults compared to these previous experiments can be attributed to our specific task properties (i.e. four instead of eight targets; see methods for details). This might have resulted in less intense learning which in turn led to less plastic changes in the adults' brain. In adults, due to their decreased level of brain plasticity, more intense stimulation of neuronal networks might be needed to reach a

substantial level of experience-dependent changes. This interpretation is in line with a previous experiment in adults demonstrating that the difficulty of a task determines whether learning induces SWA changes in the subsequent night (Schmidt et al., 2006).

An obvious limitation of the study is its cross-sectional nature. Subjects from different cohorts might per se differ with regard to individual variables (e.g. experience in using a computer mouse) and one might ask whether such cohort differences can have confounded our findings. If this was the case, learning performance should have differed between the age-groups with children performing better than adolescents and adults. We found only very subtle behavioural differences with children performing worse than adults at the end of learning whereas children and adolescents did not differ. Thus, it is unlikely that cohort specific differences affecting learning performance can explain the observed age-dependent differences in local SWA changes after visuomotor adaptation. Nevertheless, further studies are needed in which subgroups of the same cohort are tested at three different ages to fully exclude this possible confound.

Acknowledgments

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2.4. Sleep slow wave activity: towards a new marker for neural plasticity after acquired brain injury

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Submitted

Abstract

Background

Acquired brain injuries (ABI) such as traumatic brain injury (TBI) or stroke can result in motor, language or cognitive impairments. Despite that a considerable number of studies have investigated functional recovery, underlying brain reorganization remains poorly understood. Accumulating evidence indicates that plastic processes in the brain are linked to changes in electroencephalographic (EEG) slow wave activity (SWA, EEG spectral power 1-4.5 Hz) during deep sleep. In the present study, we investigated SWA in children and adolescents with ABI.

Methods

We used high-density EEG (128 electrodes) to record sleep in 22 young patients with ABI (age range: 4-16 years). We used SWA to investigate differences between patients and 52 previously measured typically developing children and adolescents (age range: 4-17 years).

Results

Individual patterns of alteration in SWA depended on nature, severity and site of the lesion. In patients with bilateral stroke, SWA was globally reduced across the entire scalp. Patients with unilateral stroke showed a local reduction in SWA over lesion areas and an increase over peri-lesional and contralateral brain areas. In patients with severe TBI, we found a reduction in SWA over the midline and an increase over lateral brain areas. We found no consistent pattern in patients with mild to moderate TBI.

Conclusions

SWA seems to be a sensitive marker for plastic processes after ABI. Reduced SWA may indicate areas of impaired neural function, whereas increased SWA might indicate "hyperplastic" areas presumably involved in reorganization processes. Improving our understanding of injury-related plasticity could optimize clinical prognosis.

Introduction

Injuries of the brain, such as traumatic brain injury (TBI) or stroke, can result in various neurological impairments. In children and adolescents as well as in adults, acquired brain injuries (ABI) have been associated with impairments in motor function, language and cognitive or emotional functioning (Kuhtz-Buschbeck et al., 2003; Amlie-Lefond et al., 2008; Beauchamp and Anderson, 2013). The extent, to which functions can recover, mainly depends on the degree of tissue damage and the preservation of neuronal circuits engaging intact brain areas to restore function (Wieloch and Nikolich, 2006). Recovery after smaller lesions is likely to involve peri-lesional areas, whereas contralateral areas become involved after larger injuries (Ward, 2005). It has been suggested that plastic processes related to brain reorganization might have similarities with plastic processes in the healthy brain, namely, experience-dependent plasticity and brain maturation (Murphy and Corbett, 2009). Investigating injury-related plastic processes in pediatric patients is particularly challenging, as one has to account for maturational differences.

Increasing evidence indicates that plastic processes in the healthy brain are linked to changes in electroencephalographic slow wave activity (SWA) during sleep (EEG spectral power 1-4.5 Hz, for a review, see (Tononi and Cirelli, 2014)). For instance, performing a visuomotor learning task prior to sleep induced a local increase in SWA during subsequent sleep over brain areas known to be involved in the learning task (Huber et al., 2004; Wilhelm et al., 2014). Brain maturation has also been related to changes in sleep SWA. For example, changes in the amount of SWA have been suggested to reflect brain development i.e., the increase in cortical gray matter volume during childhood and its decrease during adolescence (Campbell and Feinberg, 2009). Interestingly, while gray matter maturation proceeds from occipital to frontal brain regions (Shaw et al., 2008), a similar pattern was found for the localization of maximal SWA (i.e. from posterior brain regions during early childhood to anterior brain regions in late adolescence, see (Kurth et al., 2010a)). Given that SWA seems to be a sensitive method to assess plastic processes in the healthy brain, we propose to expand its use to investigate plasticity after ABI.

In the present study, we used high-density EEG to record sleep in children and adolescents with ABI. Our aim was to detect differences in SWA when comparing patients to typically developing children and adolescents of the same age. We suggest such differences to indicate injury-related plasticity.

Methods

Participants

Twenty-two pediatric patients with ABI were compared to 52 typically developing children and adolescents (age range 4-16 years). Demographic and clinical characteristics of the patients are shown in Table 1. All patients were recruited from the Rehabilitation Center for children and adolescents in Affoltern am Albis (Switzerland) between March 2012 and October 2014. Parents gave written informed consent. Patients who were able to communicate gave verbal consent. The study was approved by the local ethics committee. The typically developing children and adolescents were previously assessed (Kurth et al., 2010a; Pugin et al., 2015).

Behavioral assessment

Neurological deficits were assessed using the Pediatric version of the NIH Stroke Scale (PedNIHSS (Ichord et al., 2011)) and the Neurological Outcome Scale for Traumatic Brain Injury (NOS-TBI (McCauley et al., 2010; Wilde et al., 2010)). Assessments were conducted 1-2 days prior to the night of the sleep recording. In two patients, assessments were missing (patients 5 and 6). We retrospectively scored them, based on available reports from therapists and pediatricians. High scores indicate severe deficits. The Functional Independence Measure for children (WeeFIM (Ottenbacher et al., 1999)) assesses the need for assistance in daily life activities and was applied within 7 days before or after the sleep recordings. High scores indicate a high level of independence. All behavioral scores are listed in Table 1. We grouped patients according to injury etiology and sorted them according to impairment severity (i.e. PedNIHSS/NOS-TBI and WeeFIM scores).

Localization of the lesion sites

In all patients structural MRI scans had been collected within 6 days after brain injury. Based on visual inspection of the scans and templates of vascular territories of the brain (Kretschmann and Weinrich, 2007) damaged cortical and subcortical structures were determined by an experienced child neurologist (A.M.). We only considered cortical lesions covering the area of at least one electrode (i.e., a diameter of 1 cm). In a next step, we assigned areas of cortical damage to electrode sites using a template that was provided by an earlier study. This template was based on the co-registration of high-density EEG

electrode locations and individual MRI scans in a population of healthy children and adolescents (see (Kurth et al., 2012)). We categorized subcortical damage in 'bilaterally present', 'unilaterally present' or 'absent'.

High-density EEG recordings, data processing and analysis

Night sleep was recorded at the bedside. We used a high-density EEG system (Electrical Geodesics Inc., 128 electrodes). The net was well tolerated by all the patients. In one patient with disorders of consciousness (patients 3), we had to abort the sleep recording after approximately 6 hours because of increasing agitation. Recordings were sampled at 500 Hz and referenced to the vertex (Cz). The recorded EEG data were then band-pass filtered between 0.5 and 40 Hz and down-sampled to 128 Hz. Epochs containing artifacts were semi-automatically and visually rejected (Huber et al., 2000). Electrodes showing poor EEG signal quality were excluded. Data from all good quality electrodes above the ears were average-referenced. Missing data from excluded electrodes were interpolated using spherical linear interpolation (Wilhelm et al., 2014). Sleep stages were visually determined based on American Academy of Sleep Medicine standard criteria (20 s epochs, (Iber et al., 2007)). EEG spectral power was calculated for 20 s epochs (fast Fourier transform routine, Hanning window, average over five consecutive 4 s epochs). SWA was calculated by summing up spectral power from 1 to 4.5 Hz. As sleep is often fragmented in children with ABI (for a review see (Gagner et al., 2015)), we selected 90 epochs of maximal SWA throughout the entire night instead of using the usual first 90 epochs (90 epochs = 30 min). For further analysis, we averaged SWA across the selected epochs. We mapped SWA across the scalp including all 109 electrodes located above the ears and investigated two distinct aspects: the amount of SWA and its relative distribution over the scalp.

'Amount of SWA': We used absolute SWA values as a proxy of neural activity at each electrode in patients and typically developing children and adolescents. Absolute SWA values were log-transformed to assure normal distribution.

'SWA Topography': To display the scalp distribution of SWA irrespective of the overall amount of SWA we used relative SWA values. As previously done, we divided SWA values at each electrode by the average across all 109 electrodes (Kurth et al., 2010a).

Our goal was to detect differences in the 'Amount of SWA' and in the 'SWA Topography' when comparing patients with ABI to typically developing children and adolescents of the same age. To do so, we established norm values for different age groups:

4-8 years, 8-11 years, 11-14 years, 14-17 years. These norm values were based on group averages and variabilities.

Group average: For each electrode, we calculated the average of the 'Amount of SWA' (absolute values, see above) and the average 'SWA Topography' (relative values, see above) across typically developing children and adolescents of the same age group.

Group variability: Still within the determined age groups, we calculated standard deviations (sd) for the 'Amount of SWA' and the 'SWA Topography'.

In a next step we quantified deviations from age norms for all patients with ABI.

Deviations from age norms: For each electrode, we subtracted the respective group average from the patient's individual value and divided this difference by the group's sd [i.e., (individual value – group average)/group sd]. Values above or below 2.5sd were defined as significant deviations.

In a last step, we investigated whether significant deviations from age norms in the 'Amount of SWA' and in the 'SWA Topography' are suitable markers for neural plasticity after ABI. To this end we quantified overall deviation in the 'Amount of SWA' and in 'SWA Topography'.

Overall deviation: We summed up all electrodes showing a significant deviation from the age norm. For the 'Amount of SWA' we used negative and positive values to indicate an overall reduction and an overall increase, respectively.

We then performed a Receiver Operating Characteristic (ROC) curve analysis to evaluate sensitivity and specificity of overall deviation in the 'Amount of SWA' and in 'SWA Topography'. Finally, we used overall deviations in the 'Amount of SWA' and in 'SWA Topography' as covariates and performed a binary logistic regression to test how accurately our method detects changes in SWA related to ABI.

Results

Lesion sites

We localized damaged cortical and subcortical structures and determined lesion topographies (Table 1; Fig. 2-4: 1st column). As expected lesions were variable in size and location.

Table 1 Demographic and clinical characteristics of patients with acquired brain injury

Patient	Age, sex	Injury etiology	Affected subcortical structures	Affected cortical structures	Time since lesion	PedNIHSS/ NOS-TBI	WeeFIM
		Stroke with bilateral damage					
Pat 1	4 y, F	Shiga-like toxin-producing E. coli hemolytic-uremic syndrome, generalized edema, stroke (bil. a. cerebri anterior and media)	Bil. basal ganglia, bil. thalamus, bil. capsula interna	Bil. frontal lobe, bil. parietal lobe, bil. g. temporalis superior and medius	5.6 mths	34	18
Pat 2	7 y, F	Tumor resection (hypothalamic pilocytic astrocytoma), stroke (right a. cerebri media)	right basal ganglia, right capsula interna, left pons	Right frontal lobe, right g. temporalis medius and inferior	13.1 mths	27	18
Pat 3	12 y, M	Diabetic ketoacidosis, cerebral edema, herniation, stroke (bil. supratentorial, bil. crura cerebri, bil. capsula interna)	Bil. ncl. caudatus, bil. thalamus, corpus callosum (splenium), bil. hippocampus	Left fronto-basal, right g. cinguli	3.8 mths	25	20
		Stroke with unilateral damage					
Pat 4	4 y, F	Stroke (left a. cerebri media)	Left basal ganglia	Left g. temporalis superior; left g. frontalis inferior and medius; left g. precentralis; left g. postcentralis	2.6 mths	4	83
Pat 5	9 y, F	Stroke (left a. cerebri media and anterior)	Left basal ganglia; left thalamus; left ncl. caudatus	Left g. frontalis medius and inferior; left g. precentralis; left temporal lobe; left parietal lobe; left occipital lobe	5.2 mths	≥2*	n.a.
Pat 6	16 y, M	Stroke (left a. cerebri media)	Left basal ganglia, left thalamus, left capsual interna	Left g. frontalis medius and inferior, left g. precentralis, left gyrus centralis, left g. parietalis, left g. occipitalis, left insula	14.8 mths	≥2*	126
Pat 7	14 y, F	Right frontoparietal intraparenchymal and intraventricular hemorrhage with stroke (right capsula interna), cerebral edema	Right cortico-spinal tract, right capsula interna	Right g. frontalis medius and inferior; right g. precentralis; right g. postcentralis; right g. pareietalis superior; right precuneus	2.5 mths	2	101
Pat 8	14 y, M	Stroke (right a. cerebri media and anterior)		Right g. frontalis medialis, superior, medius and inferior; right g. precentralis; right g. postcentralis; right g. parietalis superior	1.9 mths	1	102
		Severe TBI					
Pat 9	13 y, M	TBI, intraventricular hemorrhage, subdural hematoma, contusion, cerebral edema, shearing injuries, DAI	Pons, midbrain, bil. cerebellum, bil. basal ganglia, left thalamus, corpus callosum		4.2 mths	32	18
Pat 10	14 y, M	TBI, intraventricular hemorrhage, subdural hematoma, contusion, cerebral edema, shearing injuries, DAI	Midbrain, bil. cerebellum, bil. basal ganglia, bil. thalamus, corpus callosum	Bil. temporo-polar, right g. frontalis medius and inferior	2.5 mths	28	18
Pat 11	13 y, M	TBI, intraventricular hemorrhage; subdural hematoma, cerebral edema, shearing injuries, DAI	Brainstem, midbrain, cerebellum; bil. basal ganglia; corpus callosum		4 mths	17	24

		Mild to moderate TBI					
Pat 12	9 y, M	TBI		Right g. frontalis medius	1.7 mths	2	n.a.
Pat 13	7 y, F	TBI, contusion, cerebral edema, shearing injuries, DAI	Bil. cerebellum, corpus callosum		1.5 mths	0	93
Pat 14	13 y, M	TBI, intraventricular hemorrhage, shearing injuries, DAI	Midbrain, bil. cerebellum, bil. thalamus, bil. basal ganglia, corpus callosum	Bil. frontal axonal injury	1.5 mths	0	n.a.
Pat 15	10 y, M	TBI, shearing injuries		Bil. frontal and parietal axonal injury, right g. frontalis superior, right g. temporalis medius and superior	2.1 mths	0	n.a.
Pat 16	9 y, F	TBI, contusion		Right g. frontalis superior and medius	1 mths	0	115
		Other etiologies					
Pat 17	5 y, F	Stroke	Left cerebellum		1.2 mths	1	94
Pat 18	10 y, M	Cardiac arrest, hypoxic-ischemic encephalopathy	Bil. basal ganglia		1.8 mths	1	116
Pat 19	14 y, F	Subarachnoid, intraparenchymal and intraventricular hemorrhagic stroke (cerebral arteriovenous malformation)	Left forceps major		3.8 mths	0	117
Pat 20	13 y, F	Intraventricular and intraparenchymal hemorrhagic stroke	Left forceps major, left hippocampus, left ventromedial thalamus		1.1 mths	0	117
Pat 21	13 y, M	Neuroborreliosis, meningoencephalitis	Brainstem, bil. basal ganglia		0.9 mths	0	117
Pat 22	12 y, M	Acute disseminated encephalomyelitis	Bil. thalamus, bil. cerebellum	Bil. centrum semiovale	0.8 mths	0	122

F = female; M = male; y = years; mths = months; TBI = traumatic brain injury; DAI = diffuse axonal injury bil. = bilateral; g. = gyrus; ncl. = nucleus; a. = arteria, n.a. = not administered; * = retrospective scoring; The ≥ sign indicates that the scores might underestimate deficits, as available information is incomplete

Sleep structure

Sleep structure differed between patients with ABI and typically developing children and adolescents (Table 2). As expected from previous literature, sleep was more fragmented in patients with ABI (i.e., higher amount of wake after sleep onset) (Gagner et al., 2015). We did not perform any further analysis on the structure of sleep, since our main interest was to investigate changes in SWA. We focused on two aspects of SWA: 1) the 'Amount of SWA' and 2) the 'SWA Topography' (see Methods for details).

Table 2 Sleep variables

	Patients (n=21)	Controls (n=51)	p value
TST (min)	506.5 ± 12.3	468.6 ± 9.4	0.03
N1 (%of TST)	7.8 ± 0.9	6.5 ± 0.5	0.19
N2 (%of TST)	54.0 ± 1.7	49.4 ± 0.9	0.01
N3 (%of TST)	14.1 ± 1.3	23.9 ± 0.9	<0.001
REM sleep (%of TST)	24.1 ± 1.5	20.2 ± 0.7	0.01
WASO (min)	11.3 ± 2.7	6.5 ± 0.9	0.03

Sleep variables (mean ± SEM) for patients with ABI and healthy controls, including all subjects in which sleep had been recorded throughout the entire night. TST = total sleep time; N1, N2, N3 = non-REM sleep stage 1, 2, 3; WASO = wake after sleep onset

Norm values from typically developing children and adolescents

In a first step, we investigated the 'Amount of SWA' and the 'SWA Topography' in typically developing children and adolescents. We determined group average and group variability for all age groups. Fig. 1 shows the results for the 'SWA Topography'. Looking at group averages we found the expected changes across development: From early childhood to late adolescence, the location of maximal values in the 'SWA Topography' shifted from posterior towards anterior brain areas (for details see (Kurth et al., 2010a)). When looking at group variability we found a similar topographical pattern. In other words, the variability in the 'SWA Topography' was highest in areas with maximal 'SWA Topography' values.

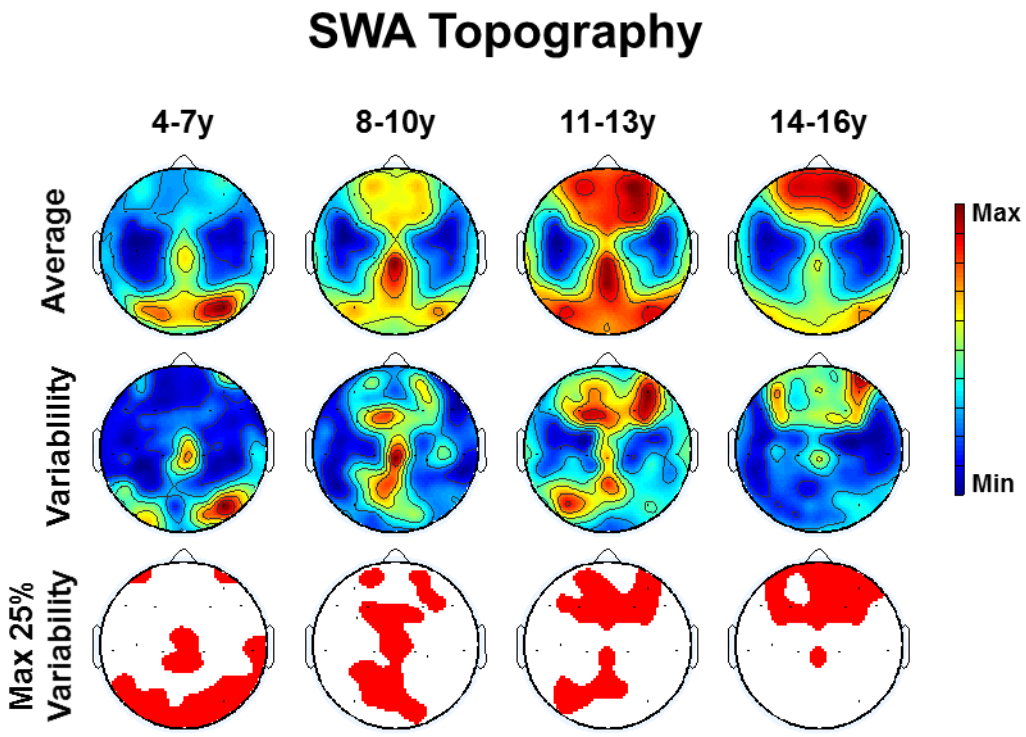

Figure 1: Results for typically developing children and adolescents: 'SWA Topography' group average (top row) and group variability (middle row). Maximal values are indicated in red, minimal values in blue. Red areas mark the top 25% values in variability (bottom row).

'SWA Topography' in patients with ABI

In a next step, we investigated the 'SWA Topography' in patients with ABI. We found both, age-specific and lesion-related aspects (Fig. 2-4, 2nd column). We determined individual deviations from age norms (Fig. 2-4, 3rd column) to quantify lesion-related changes, when controlling for age-specific patterns. Significant deviations (above/below 2.5 sd, see Methods for details) are highlighted in the 4th column of Figures 2-4. In patients with bilateral hemispheric stroke (PedNIHSS scores 25-35) the 'SWA Topography' was extensively altered (Fig. 2, patients 1-3). Patients with unilateral hemispheric stroke (PedNIHSS scores 1-4) showed a reduction in the 'SWA Topography' over lesion areas as well as over distant areas, which were mostly located in the contralateral hemisphere. We also found an increase in the 'SWA Topography' over peri-lesional and contralateral brain areas (Fig. 2, patients 4-8). In patients with severe TBI (NOS-TBI scores 17-32), we found a reduction in the 'SWA Topography' over the midline and an increase over lateral brain areas (Fig. 3, patients 9-11). We found no consistent pattern in patients with mild to moderate TBI (NOS-TBI scores 0-2; Fig. 3, patients 12-16) and in patients with various injury etiologies (PedNIHSS scores 0-1; Fig. 4, patients 17-22).

'Amount of SWA' in patients with ABI

We also investigated the 'Amount of SWA' in patients with ABI and determined individual significant deviations from age norms (Fig. 2-4, 5th column). In patients with bilateral hemispheric stroke (PedNIHSS scores 25-35), the 'Amount of SWA' was globally reduced across the entire scalp (Fig. 2, patients 1-3). In all other etiology groups, deviations in the 'Amount of SWA' were not uniform (Fig. 2, patients 4-22).

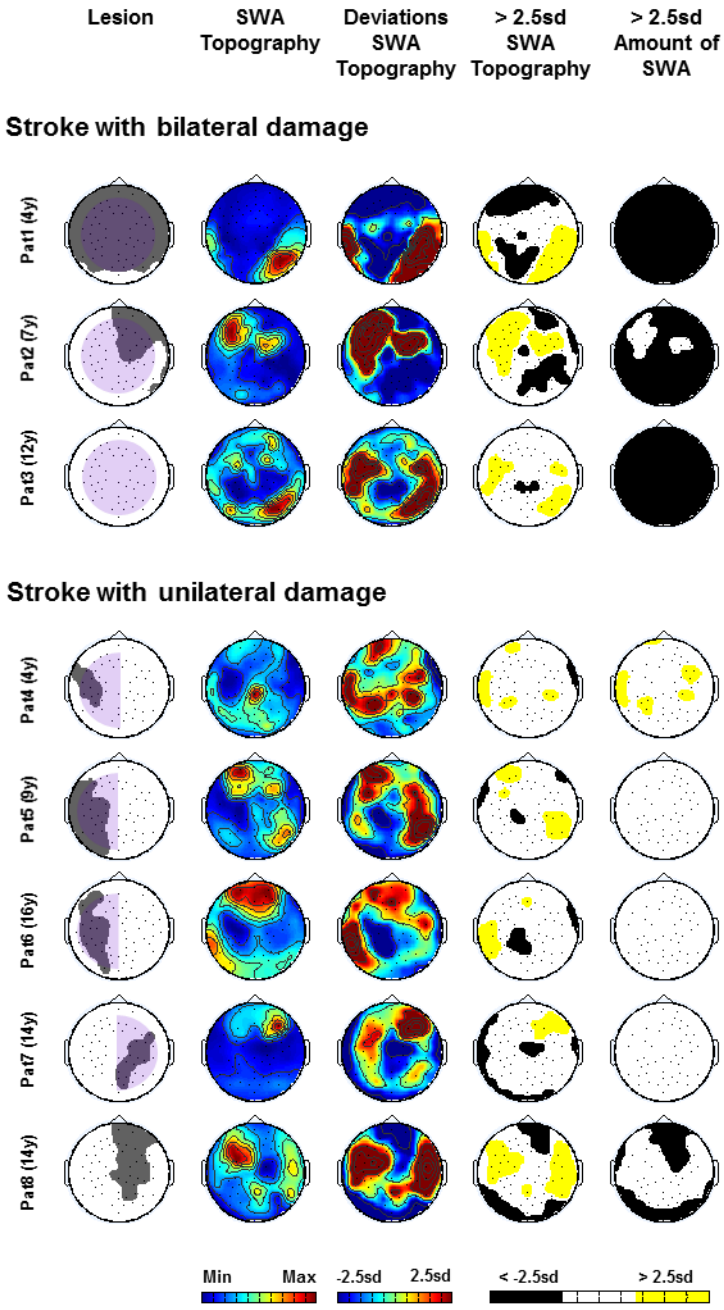

Figure 2: Results for patients with stroke: cortical (gray) and subcortical (pale purple) lesion sites (1st column), 'SWA Topography' (2nd column: maximal values in red, minimal values in blue), deviations from age norms (3rd column), significant deviations (i.e., above/below 2.5sd; 4th and 5th column).

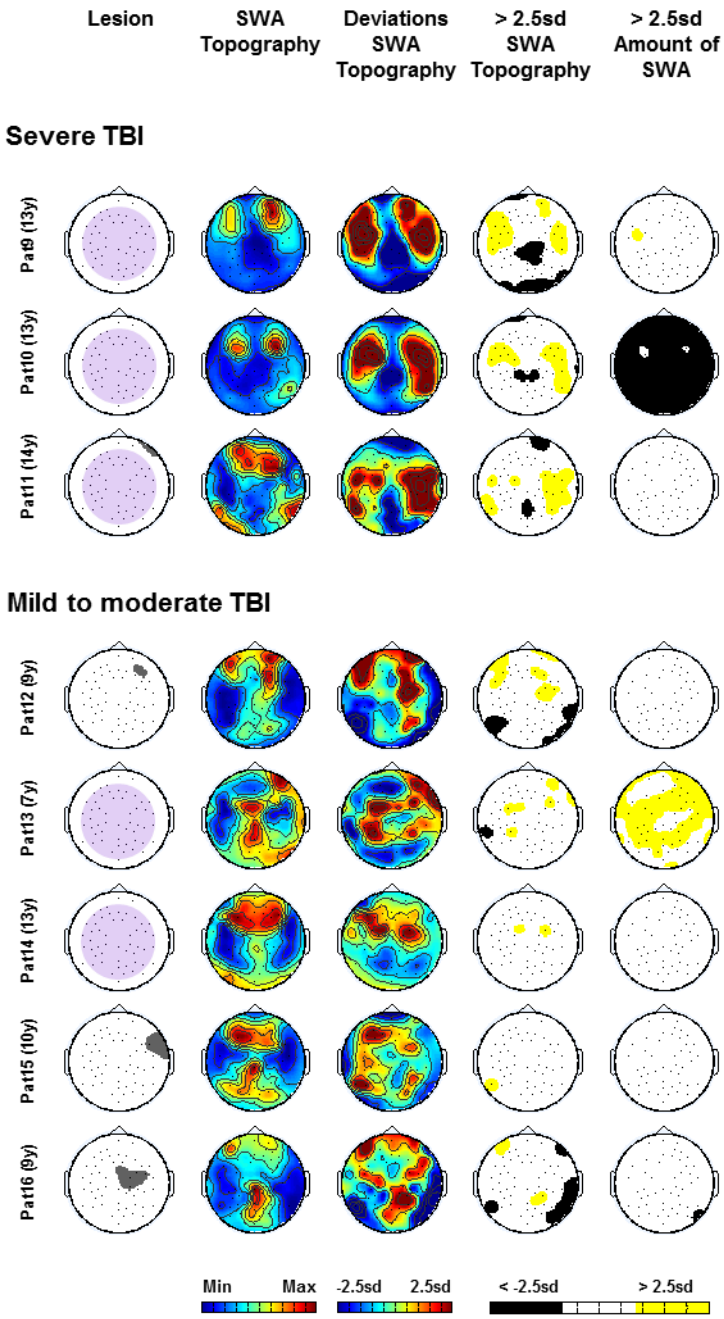

Figure 3: Results for patients with TBI: cortical (gray) and subcortical (pale purple) lesion sites (1st column), 'SWA Topography' (2nd column: maximal values in red, minimal values in blue), deviations from age norms (3rd column), significant deviations (i.e., above/below 2.5sd; 4th and 5th column).

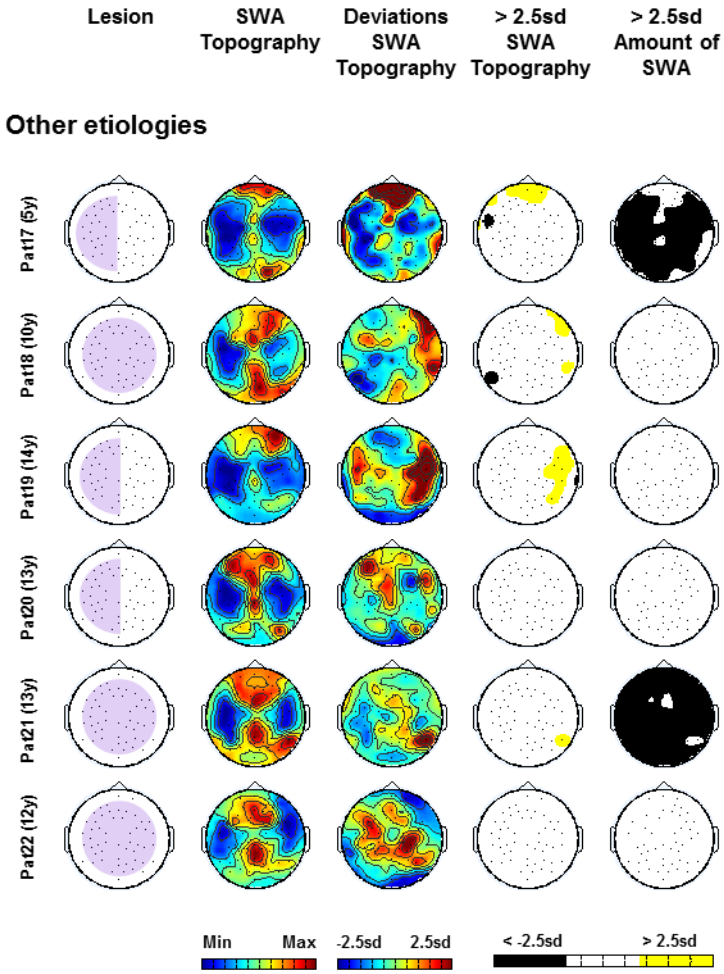

Figure 4: Results for patients with other injury etiologies: cortical (gray) and subcortical (pale purple) lesion sites (1st column), 'SWA Topography' (2nd column: maximal values in red, minimal values in blue), deviations from age norms (3rd column), significant deviations (i.e., above/below 2.5sd; 4th and 5th column).

Overall deviation in the 'Amount of SWA' and in the 'SWA Topography'

Finally, we combined the results of both, overall deviations in the 'SWA Topography' and in the 'Amount of SWA' (see Methods for details). This allowed us to determine patient's individual distances from age norms along these two dimensions (Fig. 5).

When investigating how well overall deviations in the 'SWA Topography' and in the 'Amount of SWA' could distinguish between patients and typically developing children and adolescents, ROC analyses indicated that classifications are above chance [significant areas under the curve (AUC) for the 'Amount of SWA' (AUC=0.743, p=0.001) and the 'SWA Topography' (AUC=0.893, p<0.001)]. The cut-off values that could distinguish best (i.e. with best summarized sensitivity and specificity, calculated using the Youden Index) were 1.5 for the 'Amount of SWA' (sensitivity=0.50, specificity=0.98) and 4.5 for the 'SWA Topography' (sensitivity=0.77, specificity=0.96). In other words, an overall deviation in the 'Amount of SWA' in more than one electrode was highly specific for patients with ABI (i.e. rarely present in typically developing children and adolescents), but less sensitive (i.e. not only the typically developing children but also many patients showed deviations in less than two electrodes). We performed a binary logistic regression analysis to estimate the predictive value of combined overall deviations in the 'SWA Topography' and in the 'Amount of SWA' with respect to the categorization into patients or typically developing children and adolescents. The results showed a good fit of the model (Nagelkerke R^2= 0.727) and a 92% accuracy of classification. This implies that overall deviations in the 'SWA Topography' and in the 'Amount of SWA' provide complementary information to classify patients.

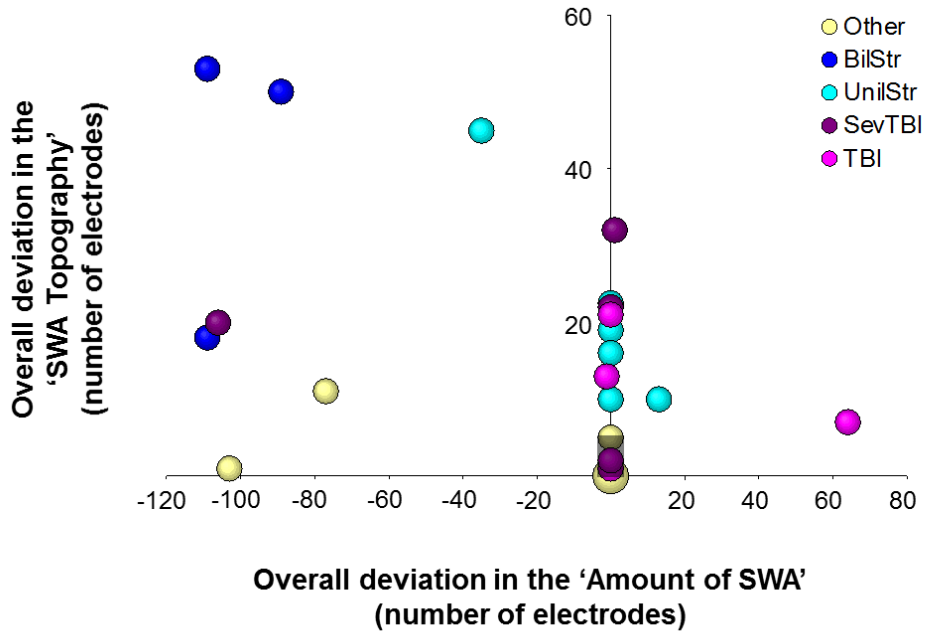

Figure 5: Patient's overall deviation in the 'Amount of SWA' (x-axis) and in the 'SWA Topography' (y-axis). Etiology groups: TBI, severe TBI (SevTBI), stroke with unilateral (UnilStr) or bilateral damage (BilStr), Other. The shaded area represents the 95th percentiles for typically developing children and adolescents ('Amount of SWA': 0; 'SWA Topography': 5).

Discussion

In the present study, we propose a novel approach to investigate neural plasticity after ABI. We analyzed the 'Amount of SWA' and 'SWA Topography' in pediatric patients with ABI and determined deviations from age norms. Interestingly, the pattern of these deviations seemed to depend on nature, size and location of the lesion, in particular for patients with stroke and severe TBI.

Patients with stroke: In patients with bilateral damage, we found an overall reduction in the 'Amount of SWA' and global changes in the 'SWA Topography'. This might reflect a general impairment of brain network function. In patients with unilateral damage, we found a local reduction in the 'SWA Topography' over lesion areas, which might reflect an impairment of region-specific brain network function. The intriguing finding that also distant brain areas showed a reduction in the 'SWA Topography' might be explained in the context of diaschisis. Diaschisis has been defined as neural hypoactivity in anatomically intact distant brain areas, directly caused by a focal injury (Carrera and Tononi, 2014). The lack of synaptic input from the lesion area is assumed to result in a loss of excitability also referred to as 'functional stillstand' (von Monakow, 1914). According to Carrera and Tononi (Carrera and Tononi, 2014) diaschisis should tend to disappear over time. However,

we found a persistent diaschisis-like pattern in all our patients with unilateral damage. Four of them were in a sub-acute and one in chronic stage. To our knowledge, diaschisis has never been investigated during sleep, thus we cannot differentiate between the possibility that our findings depend on the pediatric patient population or on the sleep condition. Besides areas of local reduction in the 'SWA Topography', patients with unilateral damage also showed local increases in the 'SWA Topography', mainly over peri-lesional and contralateral brain areas. Those areas are known to play a crucial role in function recovery after stroke (Ward, 2005). We suggest that increased SWA may indicate "hyperplastic" brain areas involved in brain network restoration and/or recruitment of alternative networks. Patients with severe TBI: Patients with severe TBI showed strikingly similar changes in their 'SWA Topographies'. They all showed a reduction over the midline and an increase over lateral brain areas. This pattern is likely to reflect a dysfunction of the same network caused by axonal lesions as none of the patients with severe TBI showed cortical damages over this midline. Patients with mild to moderate TBI showed a highly variable pattern of alterations in the 'SWA Topography'. This is not surprising, given the large variety of lesions. Currently, many aspects of how TBI affects structural and functional networks and how such alterations are related to neurological deficits remain unclear. Multimodal approaches, combining structural and functional information could improve this understanding (Caeyenberghs et al., 2013; Sharp et al., 2014; Dean et al., 2015; Dennis et al., 2015).

Results from other patients were less conclusive but still indicated deviations from age norms in most of the cases.

Overall, our approach appears to provide sensitive and specific markers for injury-related neural plasticity in children and adolescents with ABI. Our results are in agreement with two previous studies, which investigated neural plasticity in adult patients with stroke by means of high-density EEG recordings during sleep (Sarasso et al., 2014; Poryazova et al., 2015). Cross-sectional comparisons between acute patients and healthy controls as well as longitudinal comparisons in chronic patients undergoing speech therapy could point out local differences in SWA. In contrast to these group comparison studies, we chose to investigate alterations in SWA at the single-subject level. We consider our approach to be the preferable choice when investigating heterogeneous patient groups such as our group of children and adolescents with ABI (i.e. different states of brain maturation and different injuries).

Our study also has some limitations. At this point, we cannot answer the question, whether ongoing brain maturation influences neural plasticity after ABI. Our patient group was too heterogeneous and too small to allow any assumption in this direction. To pursue this objective we would need measurements from multiple patients of different ages, with similar brain injuries. Longitudinal measurements in the course of rehabilitation therapy could provide information about within-subject plastic changes. The yet missing link between neural reorganization and functional recovery could also be addressed in this context.

Potential clinical implications from our study are that beyond complementary diagnostic and prognostic information, the localization of hyperplastic brain areas could provide a basis for novel therapeutic interventions such as brain stimulation. Targeting orchestrating reorganization areas might boost plasticity in the entire network inducing something like an 'inverse diaschisis' (i.e. increased local activity due to repeated synaptic input).

Conclusions

This is the first study investigating neural plasticity in children and adolescents with ABI by means of high-density EEG recordings during sleep. Such recordings are easy to apply in the clinical setting, also in critically ill and non-cooperative patients. SWA seems to be a sensitive marker for plastic processes after ABI. Individual patterns of alterations in SWA might point out lesion-related areas of impaired neural function and/or "hyperplastic" areas presumably involved in network reorganization. Such functional measurements could complement the structural information provided by MRI. Improving our understanding of neural plasticity after ABI could not only assist clinical diagnosis and prognosis but may also guide the development of novel therapeutic interventions (e.g. brain stimulation).

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2.5. High-density electroencephalographic recordings during sleep in children with disorders of consciousness

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Under review

Abstract

Introduction: A large number of studies have investigated neuronal correlates of consciousness in adults. However, knowledge about brain function in children with disorders of consciousness (DOC) is very limited. We suggest that EEG recordings during sleep are a promising approach. In healthy adults as well as in children, it has been shown that the activity of sleep slow waves (EEG spectral power 1-4.5 Hz), the primary characteristic of deep sleep, is dependent on use during previous wakefulness. Thus the regulation of slow wave activity (SWA) provides indirect insights into brain function during wakefulness.

Methods: In the present study, we investigated high-density EEG recordings during sleep in ten healthy children and in ten children with acquired brain injury, including five children with DOC and five children with acquired brain injury without DOC. We used the build-up of SWA to quantify SWA regulation.

Results: Children with DOC showed a global reduction in the SWA build-up when compared to both, healthy children and children with acquired brain injury without DOC. This reduction was most pronounced over parietal brain areas. Comparisons within the group of children with DOC revealed that the parietal SWA build-up was lowest in patients showing poor outcome. Longitudinal measurements during the recovery period showed an increase in parietal SWA build-up from the first to the second sleep recording.

Conclusions: Our results suggest that the reduced parietal SWA regulation may reflect a disorder-specific alteration in brain function in children with DOC. This interpretation is in agreement with the currently proposed model for consciousness claiming that frontoparietal networks play a critical role in DOC. In the future, the regulation of SWA might be used as a complementary assessment in adult and pediatric patients with DOC.

Introduction

After severe traumatic or non-traumatic brain injury surviving patients often show disorders of consciousness (DOC). Traditionally, DOC are categorized into coma, in which patients are completely unarousable and unresponsive, vegetative state (VS) defined by the re-emergence of spontaneous eye-openings and minimally conscious state (MCS), in which patients start to show non-reflexive responses to stimuli. In clinical practice, the gold standard for the diagnosis of DOC are behavioural assessment scales like the Coma Recovery Scale - Revised (Giacino et al., 2004). Such assessments are very challenging as a lack of motor functions, receptive aphasia or fluctuations in arousal might lead to false negative results and consequently to a misdiagnosis (Giacino et al., 2009). Hence, much effort has focused on the development of complementary methods to detect neuronal correlates of consciousness. Functional neuroimaging and electrophysiological measurements in patients with DOC and healthy subjects have provided novel insights into neurobiological aspects of consciousness (for a review see (Giacino et al., 2014)). While functional MRI and PET may not always be available in the clinical setting, EEG measurements can easily be performed at the bedside. Another advantage of EEG is the possibility of long-duration measurements including sleep. Such long-duration measurements are especially convenient, when assessing patients with DOC, as these patients typically show frequent fluctuations in the level of arousal (Forgacs et al., 2014).

In patients with DOC the presence or absence of normal sleep features such as different sleep stages, sleep spindles and sleep slow waves has been associated with behavioural outcome and was hypothesized to reflect global functional brain integrity (Cheliout-Heraut et al., 2002; Landsness et al., 2011; Cologan et al., 2012; Malinowska et al., 2013; de Biase et al., 2014; Rossi Sebastiano et al., 2014; Avantaggiato et al., 2015). More specifically, sleep spindles and sleep slow waves are known to involve thalamocortical and corticocortical circuits (e.g., (Schabus et al., 2007; Riedner et al., 2011)) and thus, might be suitable markers for preserved thalamocortical and frontoparietal connectivity, which in turn has been related to consciousness (Laureys and Schiff, 2012).

While, in recent years, many complementary neurophysiological methods for the assessment of DOC have been investigated in adults, very few have been applied to children. In fact, the only studies reporting more than single cases, investigated the presence or absence of sleep stages and sleep spindles in children with DOC (Cheliout-Heraut et al., 2002; Avantaggiato et al., 2015). Compared to adult patients, pediatric patients hold the additional

difficulty that differences in brain activity result not only from brain injury but also depend on brain maturation. During development, the brain undergoes critical anatomical and functional maturation processes, such as synaptic pruning and changes in functional network efficiency (e.g., (Huttenlocher and Dabholkar, 1997; de Bie et al., 2011)). Therefore, if applied in children, neurophysiological correlates of consciousness have to account for maturational differences.

EEG recordings during sleep might be a promising approach to investigate patients with DOC. It has been shown that the activity of sleep slow waves (EEG spectral power 1-4.5 Hz), the primary characteristic of deep sleep (Borbely and Achermann, 2000), is dependent on use during previous wakefulness This use-dependent regulation of slow wave activity (SWA) is best seen on a local level. Studies investigating SWA across the scalp found local increases over brain areas that had been used extensively (Kattler et al., 1994) or were involved in a learning task, prior to sleep (Huber et al., 2004; Wilhelm et al., 2014). Accordingly, when the use of specific brain areas was prevented (i.e., arm immobilization) SWA was locally decreased (Huber et al., 2006).Thus, the local regulation of SWA might serve as an indirect measure of the activity level of specific brain areas during wakefulness. Interestingly, the scalp distribution of SWA also shows regional differences in the course of development (Kurth et al., 2010a). From early childhood to late adolescence the location of maximal SWA undergoes a shift from posterior towards anterior brain regions. These changes were proposed to reflect cortical brain maturation.

In our study, we recorded sleep in ten children with acquired brain injury (five with DOC, five without DOC) using high-density EEG. We investigated the regulation of SWA across the scalp and compared children with DOC to both, healthy children and children with acquired brain injury without DOC. We hypothesized that local differences in sleep SWA regulation might reveal brain areas playing a critical role in pediatric DOC. Longitudinal measurements in children with DOC could further support our hypothesis and might even provide prognostic information.

Materials and methods

Patients

Ten children with acquired brain injury due to traumatic brain injury or stroke participated in the study, including five children with DOC (mean age 10 years, SD 4.3 years, range 4-14

years of age, two girls and three boys) and five age- and gender-matched children with acquired brain injury without DOC (mean age 10 years, SD 4.3 years, range 4-14 years of age, two girls and three boys). Demographic and clinical characteristics of the patients with DOC are shown in Table 1. Medication is documented in Supplementary Table 1. All patients were recruited from the Rehabilitation Center for children and adolescents in Affoltern am Albis in Switzerland over a period of three years. The participation rate was high. During the recruitment period, six patients with DOC were admitted to the rehabilitation center. Five of them participated in the study. Parents from all patients gave written informed consent. Patients with acquired brain injury without DOC gave verbal consent. The study was approved by the local ethics committee.

Healthy subjects

We selected ten age- and gender-matched healthy children (mean age 10 years, SD 4.1 years, range 4-14 years of age, four girls and six boys) from earlier studies (Kurth et al., 2010a; Pugin et al., 2015).

Behavioural assessment

Patients with DOC were assessed by a trained neuropsychologist (ALM with clinical training at the Coma Science Group in Liège, Belgium) using the Coma Recovery Scale-Revised (Giacino et al., 2004). This scale provides scoring rules for observable behaviour during auditory, visual, motor, oromotor, communication and arousal testing and categorizes patients into VS, MCS and emergence from MCS. During the week of the sleep recording, the Coma Recovery Scale-Revised was performed daily. The diagnosis was based on the best result (Table 1).

High-density electroencephalographic sleep recordings

Night sleep was recorded at the bedside in ten patients who had been admitted to the rehabilitation center after acquired brain injury. In four out of the five patients with DOC, a second sleep recording was performed at a later time point (see Table 1). We used a high-density EEG system (Electrical Geodesics, 128 electrodes) including two external electrodes for the submental EMG. Recordings were sampled at 500 Hz and referenced to the vertex (Cz). Offline the EEG data was band-pass filtered between 0.5 and 40 Hz and the EMG data between 20 and 40 Hz. All data were down-sampled to 128 Hz. EEG spectral power was calculated for 20 s epochs (fast Fourier transform routine, Hanning window, average over five consecutive 4 s epochs). Epochs containing artefacts were semi-automatically and visually rejected (Huber et al., 2000). Electrodes showing poor EEG signal quality were excluded. Data from all good quality electrodes above the ears were average-referenced e.g., (Kurth et al., 2010a)).

Analysis of sleep recordings

Sleep stages for 20 s epochs were visually determined based on standard criteria provided by the American Academy of Sleep Medicine (Iber et al., 2007). Fig. 1 and Supplementary Fig. 1 show individual sleep scorings and SWA (EEG spectral power 1-4.5 Hz) over a central derivation across the night. For our analysis, we chose to use the build-up of SWA as a measure for the use-dependent SWA regulation. In healthy adults and children, the build-up of SWA is commonly calculated for the first non-REM sleep episode, including approximately 30 min of non-REM sleep (e.g., (Bachmann et al., 2012; Tarokh et al., 2012)). As patients with DOC typically show fragmented sleep conditions, we developed the following approach to quantify multiple shortened periods of SWA regulation: After artefact removal, we calculated the mean SWA for 1-min epochs across the entire night, including all sleep stages. Next, we identified episodes of SWA build-up, which had a duration of at least 9 min and selected the four episodes of maximal SWA build-up (Fig. 2 and Supplementary Fig. 2). We determined the threshold of 9 min based on the maximal SWA build-up duration reached in the patient showing the highest sleep fragmentation (Fig.1 and Fig. 2: DOC 4). We used four episodes to increase the amount of data included in the analysis. In one patient (DOC 3) we only determined three episodes of SWA build-up due to a shortened total sleep time (< 3 h). We quantified the build-up of SWA using a linear fitting approach and averaged the values across all episodes within individuals. Missing values from electrodes excluded because of poor data quality were interpolated.

We mapped the SWA build-up including all electrodes above the ears (109 electrodes) for all patients and healthy subjects (patients with DOC and the five closest age-matched healthy subjects shown in Fig. 3). We used relative values to investigate topographical aspects irrespective of absolute SWA build-up differences. These relative SWA build-up values were obtained by calculating the ratio between the SWA build-up from each electrode within the topography and the average across the topography. In order to investigate differences within the group of patients with DOC, we calculated age-normalized relative SWA build-up values. For this purpose, we calculated the ratio of the relative SWA build-up between individual patients with DOC and the mean over their respective matched healthy subjects (Fig. 5).

For our analysis, we decided to use the build-up of SWA rather than SWA itself. This decision was based on the assumption that in patients with acquired brain injury SWA might be confounded by the presence of lesion-related slow-oscillations arising as a result of cortical deafferentation (Steriade et al., 1993c; Lemieux et al., 2014). Contrary to sleep slow waves we hypothesized that these pathophysiological slow oscillations are independent of sleep regulatory processes. Thus, we investigated the topographical contrast between relative SWA across the night and relative SWA build-up in all patients and healthy subjects. We calculated the ratio between relative SWA and SWA build-up and determined individual clusters of four electrodes showing maximal values (i.e., higher relative SWA than SWA build-up). We then mapped the topographical cluster distribution for patients and healthy subjects (Supplementary Fig. 3). The clusters from healthy subjects were all located over frontocentral brain areas. Conversely, the clusters from patients with acquired brain injury were widely scattered over the scalp. Thus, the relationship between SWA and SWA regulation indeed was changed in patients. This finding supports our assumption of confounded SWA after brain injury and confirms our choice to use the build-up of SWA for our analysis.

Statistics

Topographical group comparisons of the SWA build-up between patients with DOC and patients with acquired brain injury without DOC were performed using an unpaired two-tailed t-test for each electrode ($P < 0.05$). Group comparisons between patients with DOC and healthy subjects were performed using a combinatorial approach. We had two age- and gender-matched healthy subjects for each patient with DOC. This resulted in 32 possible group combinations for the healthy control group. We performed 32 two-tailed unpaired t-tests for each electrode. Topographical differences were reported significant when the 95% confidence interval of the p-value was below 0.05.

Results

At the time of the first sleep recording, four patients were in an MCS, one patient had emerged from MCS two days prior to the sleep recording (Table 1).

Table 2 Demographic and clinical characteristics of patients with DOC

Patient: Age, gender	Aetiology. pathology	S1 time since insult	S1 CRS-R diagnosis (total score)	Time interval S1 – S2	S2 CRS-R diagnosis (total score)
DOC 1: 4 y, F	Shiga-like toxin-producing E. coli hemolytic-uremic syndrome, stroke (bilateral anterior and middle cerebral artery including basal ganglia)	5 months	MCS (9)	16.1 months (S2 not analyzed due to epileptic activity)	MCS (9)
DOC 2: 7 y, F	Tumor resection (hypothalamic pilocytic astrocytoma), stroke (right basal ganglia, right internal capsule, left pons)	1.1 years	MCS (16)	Deceased	Deceased
DOC 3: 12 y, M	Diabetic ketoacidosis, generalized cerebral oedema, brain herniation, stroke (bilateral basal ganglia, bilateral internal capsule, bilateral cerebral crus, bilateral thalamus)	4 months	Emergence from MCS (22)	1.5 months	Fully conscious
DOC 4: 13 y, M	Traumatic brain injury, right frontoparietal and frontotemporal subdural haematoma, contusion (right cerebellum), cerebral oedema (midbrain, basal ganglia), haemorrhage (corpus callosum, brain stem), shearing injuries (subcortical, basal ganglia)	4 months	MCS (11)	4 months	Emergence from MCS (18)
DOC 5: 14 y, M	Traumatic brain injury, multiple shearing injuries (right corpus callosum, right basal ganglia, right thalamus, right midbrain), bilateral frontopolar and right frontobasal contusions	3 months	MCS (13)	4.9 months	Fully conscious

S1 = sleep recording first night; S2 = sleep recording second night; CRS-R = Coma Recovery Scale Revised; F = female; M = male

We found different sleep stages to be present in all of them (Fig. 1). However, when compared to healthy subjects we found major differences in terms of sleep fragmentation and duration of waking after sleep onset (see also Supplementary Fig. 1). We developed an adapted calculation for the build-up of SWA to quantify SWA regulation (see Materials and methods for details).

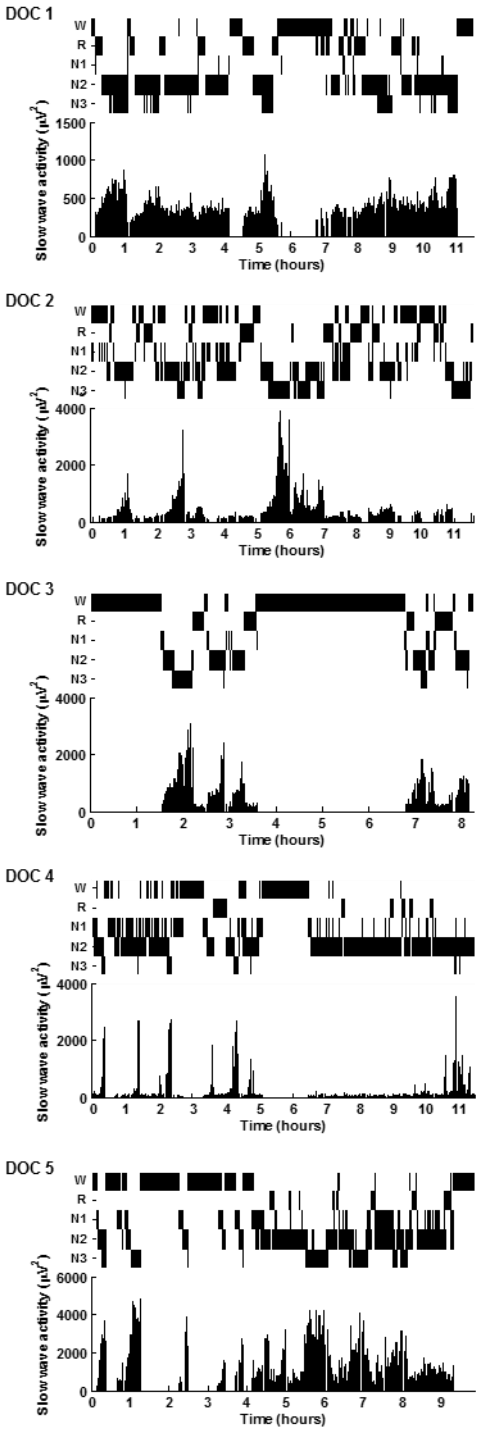

Figure 1 Hypnogram and slow wave activity (SWA) time course across the night for five patients with DOC. The sleep scoring includes wake (W), rapid eye movement sleep (R), NREM sleep stages N1, N2 and N3. SWA is shown for a frontal derivation. See Supplementary Fig. 1 for the hypnogram and SWA time course for the other groups of subjects.

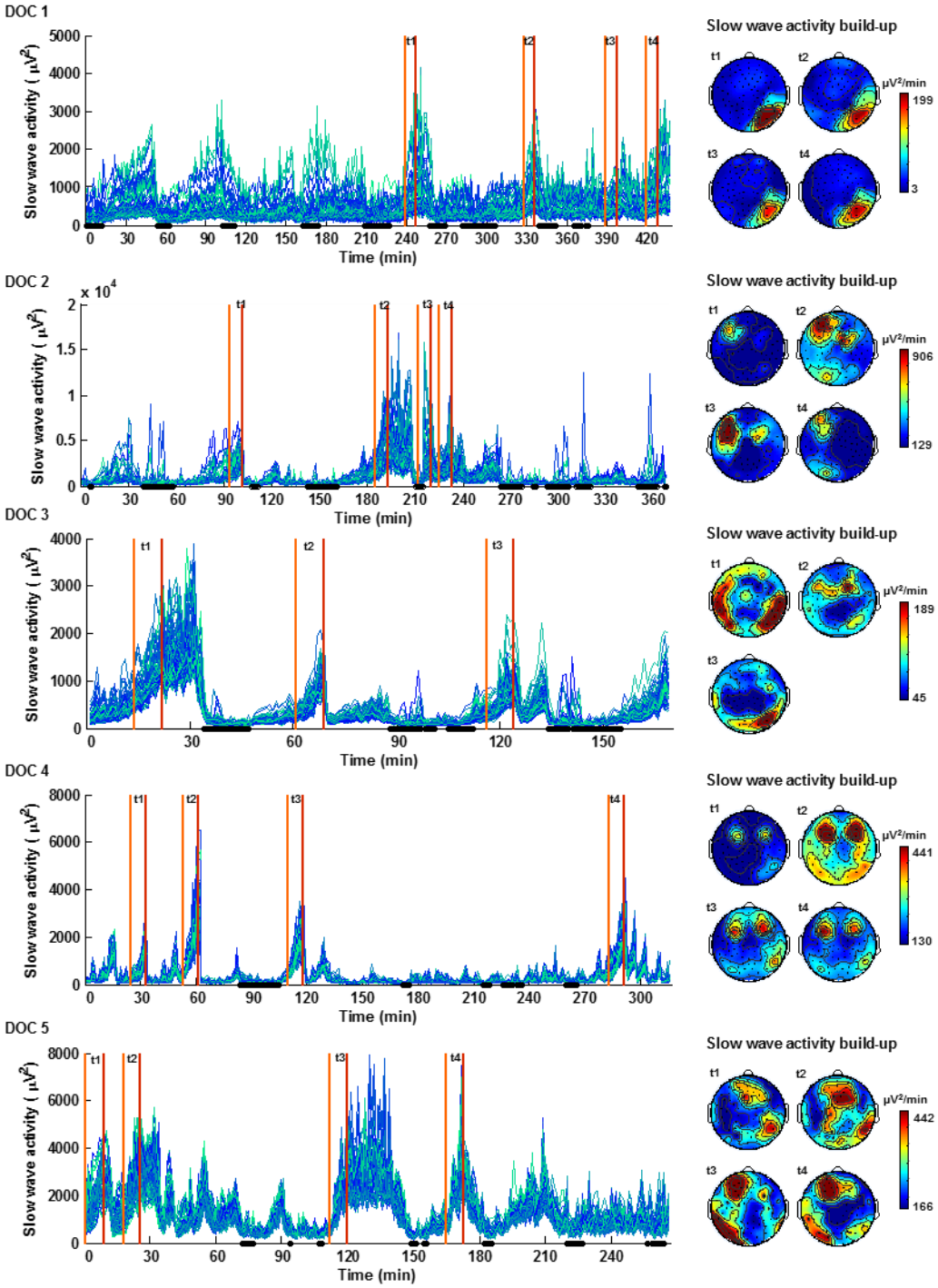

Figure 2 SWA time course and scalp distribution of SWA build-up for five patients with DOC. SWA is shown across 1 min epochs. Each line represents the SWA time course for one electrode. Only artefact free sleep epochs were included. Black dots on the bottom line indicate epochs of REM sleep. Vertical lines in orange and magenta mark beginning and end of the four 9 min episodes of maximal SWA build-up. Topographical plots (right) show the distribution of the SWA build-up for the four determined episodes. Color coding was individually scaled in order to optimize the visualization of topographical patterns (maximal values in red, minimal values in blue). See Supplementary Fig. 2 for same plots of the other groups of subjects.

In healthy children, we found expected age-specific topographical distributions with a maximal SWA build-up over more posterior brain areas in younger children and more anterior brain areas in older children respectively (Fig. 3, lower row; see Kurth *et al.* , 2010 for a detailed description of typically developing children and adolescents). In a first step we assessed group differences on a global level. Children with DOC showed severely altered patterns (Fig. 3, upper row) and lowest SWA build-up values (Fig. 4, upper row). Both group comparisons (i.e., patients with DOC vs. patients with acquired brain injury without DOC and patients with DOC vs. healthy subjects) showed significant differences over large areas of the brain. In a next step we investigated local differences between the groups using normalized values of SWA. The relative SWA build-up in patients with DOC was significantly lower over parietal brain areas (Fig. 4, lower row) when compared to the two other groups. Additionally, we found a small frontal cluster in healthy subjects and a single frontal electrode in patients with acquired brain injury without DOC showing significantly higher values than in patients with DOC.

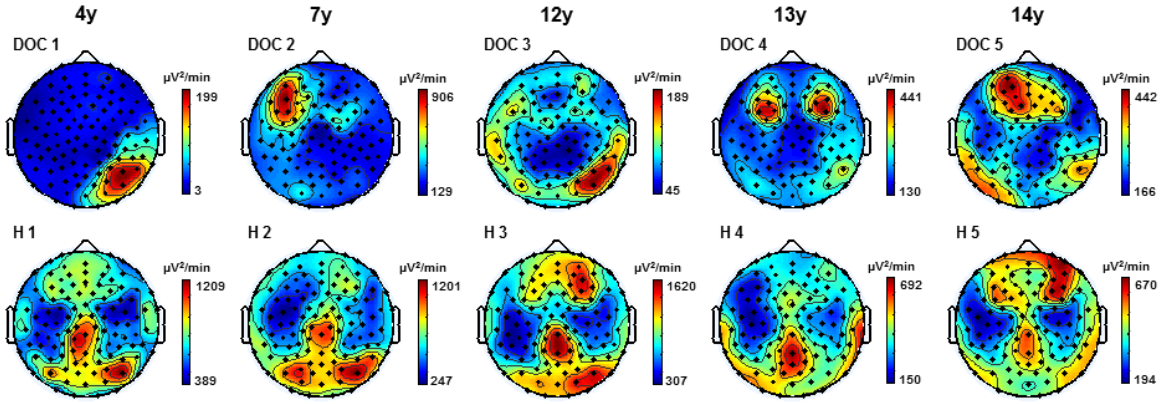

Figure 3 Scalp distribution of SWA build-up for five patients with DOC and five age- and gender-matched healthy subjects (H). Age is indicated in years (y).

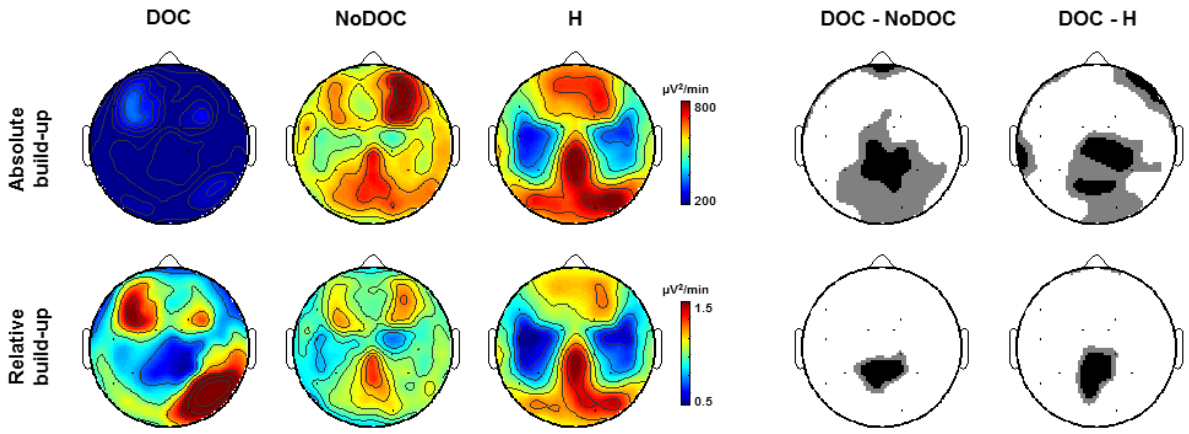

Figure 4 Left: Scalp distribution of absolute (top row) and relative (bottom row) SWA build-up for patients with DOC, patients with acquired brain injury but without DOC (NoDOC) and healthy subjects (H). Values were color coded using the same scale for all three groups. Right: Significant group differences in absolute (top row) and relative (bottom row) SWA build-up (in black p<0.01, in grey p<0.05).

In a final step, using age-normalized relative SWA build-up values, we investigated differences in the parietal electrode cluster between individual patients with DOC. We found lowest values in patients showing poor outcome (Fig. 5, Table 1). Longitudinal changes in the three recovering patients showed an increase in age-normalized relative SWA build-up values from the first to the second sleep recording. The second sleep recording from patient DOC 1 had to be excluded from the analysis, due to continuous epileptic activity.

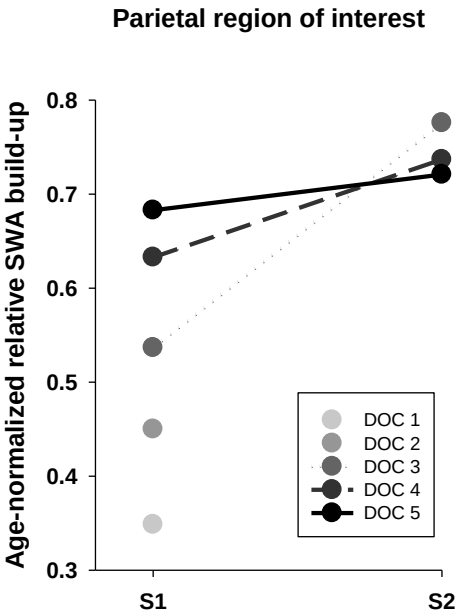

Figure 5 Age-normalized relative SWA build-up in the parietal electrode cluster for patients with DOC: five patients at the time of the first sleep recording (S1) and three patients at the time of the second sleep recording (S2). Age-normalized values were obtained by calculating the ratio between individual patients and the mean over their respective matched healthy subjects.

Discussion

In the present study, we investigated topographical patterns of SWA regulation in children with DOC. This patient group showed a widespread reduction of SWA build-up when compared to both, healthy subjects and patients with acquired brain injury without DOC. This indicates a global dampening of regulatory aspects of sleep in patients with DOC. The topographical distributions of relative SWA build-up revealed local differences. Patients with DOC showed a reduced relative SWA build-up over parietal brain areas and over a smaller frontal brain area when compared to the two other groups. As no patient had a local damage in parietal brain areas, we suggest that the parietal reduction in the SWA build-up does not simply reflect brain damage but rather indicates a disorder-specific alteration in brain function. Our results are in agreement with a recent high-density EEG study that found resting state spectral power measures over parietal brain regions to be sensitive indices of consciousness in adults (Sitt et al., 2014). Also functional MRI and PET studies identified the parietal cortex as being a brain area critically involved in DOC (for a review see (Laureys and Schiff, 2012)). Thus, the local reduction of SWA regulation over parietal brain areas might indeed reflect impaired brain activity during previous wakefulness.

Considering the current state of research in the field, Laureys and Schiff (Laureys and Schiff, 2012) proposed a model of consciousness accounting for transitions across the continuum of DOC. They suggest frontoparietal and thalamocortical networks to be modulated by a mesocircuit including the frontal cortex, the thalamus and parts of the basal ganglia. Interestingly, frontoparietal and thalamocortical networks are also known to be involved in the generation and propagation of sleep slow waves (Massimini et al., 2004; Murphy et al., 2009). Thus, SWA regulation over parietal brain areas might reflect the functional integrity of a common network and, therefore, might be indirectly linked to DOC.

In our pediatric patient population, the reduced relative SWA build-up over parietal brain areas was a consistent finding across a wide age range (4 to 14 years of age). Thus, we hypothesize that functional networks underlying consciousness might be similar throughout childhood and adulthood. Indeed, functional networks were found to be already present in childhood, yet showing an immature organization (Fair et al., 2007; de Bie et al., 2011). Consequently, we suggest that the proposed network model of consciousness may also be applied to children, even though network connectivity is likely to show age-dependent differences. Furthermore, the fact that all children with DOC had lesions in the area of the

basal ganglia (see Table 1) indicates that the proposed mesocircuit might also play a critical role in pediatric patients with DOC.

In our study, we focussed on regulatory aspects of brain activity during sleep. A previous study in adult patients with DOC used the decline of SWA across the night as a maker for sleep regulation (Landsness et al., 2011). However, we did not see this typical trajectory of SWA in our young patients (see Fig. 1). Possible reasons would be daytime sleep or night sleep fragmentation. For our analysis, we used the build-up of SWA to quantify use-dependent SWA regulation. We developed an adapted calculation to account for sleep fragmentation, including four episodes of 9 min SWA build-up (see Methods for details). This was an optimal trade-off between using episodes of maximal possible duration and including multiple episodes to obtain a total duration of approximately 30 min (i.e., the same amount of data commonly used in healthy subjects). However, in other populations, the optimal trade-off might be different (e.g., three episodes of 12 min SWA build-up). Nevertheless, the basic principle to determine this optimum should stay the same. In our population, we further tested whether the different episodes included in the analysis were comparable. We randomly selected one out of the four 9 min episodes in each patient and healthy subject and calculated group differences. Repeating this procedure we consistently obtained significant group differences over parietal brain areas (data not shown). Thus, averaging the SWA build-up across episodes seems to be appropriate.

The local regulation of SWA has been related to learning tasks prior to sleep (Huber et al., 2004; Wilhelm et al., 2014). The fact that learning is known to induce plastic changes (for a review see (Dayan and Cohen, 2011)) supports a current hypothesis that SWA regulation might not just reflect brain use but more specifically, brain plasticity (Tononi and Cirelli, 2014). Neuronal plasticity is also known to play a critical role in brain reorganization processes after acquired injury (Wieloch and Nikolich, 2006). In this latter context, SWA regulation in patients with acquired brain injury might indicate local capacities for functional or even structural brain reorganization. The finding that in patients with DOC a higher parietal SWA build-up was associated with a better outcome would support this assumption since a higher plastic capacity should favour recovery.

A limitation of our study is the small sample size. However, our findings were consistent across patients with different aetiologies and across a wide age range (4-14 years) suggesting a robust pattern. Further studies investigating brain activity in children with DOC are needed to improve our understanding of the pathophysiological mechanisms. Such knowledge is

crucial to provide accurate diagnosis and prognosis and to guide and evaluate potential treatments.

From a clinical perspective, our results suggest that high-density sleep recordings might be a suitable method to complement behavioural assessments in children with DOC. Residual parietal SWA regulation might provide additional diagnostic as well as prognostic information. On the one hand, this measure might reflect functional network integrity related to consciousness levels, on the other hand, it might indicate plastic capacity related to outcome. Potential therapeutic implications could imply local brain stimulation over parietal regions using, for example, transcranial direct current stimulation (tDCS) during wakefulness or transcranial oscillatory direct current stimulation (toDCS) during sleep. In adult patients with DOC, tDCS over the left dorsolateral prefrontal cortex improved behavioural performance (Thibaut et al., 2014). In children with attention-deficit hyperactivity disorder (ADHD), frontal toDCS during sleep increased the activity of sleep slow oscillations and improved memory performance (Prehn-Kristensen et al., 2014).

With the traditionally available diagnostic measures prognostic information regarding recovery in patients with DOC, is still extremely difficult to obtain. Complementary diagnostic and prognostic information would not only be beneficial for health professionals to plan rehabilitation treatment but also meet needs of parents. The fact that despite the stressful situation, most of our patients' parents agreed to participate in the study, speaks for this assumption.

Conclusion

To our knowledge, this is the first study investigating topographical differences in brain activity between children with acquired brain injury and DOC, children with acquired brain injury without DOC and healthy children. We used the regulation of SWA as an indirect measure for brain activity during previous wakefulness. Our results suggest parietal brain areas to play a critical role in pediatric DOC. This is in agreement with the currently proposed model of consciousness in adults. From a clinical perspective, this study is a first step towards complementary methods for diagnosis, prognosis and novel therapeutic interventions in children with DOC. Given the small number of pediatric patients, future studies should attempt to involve multiple clinical and research centers.

Acknowledgements

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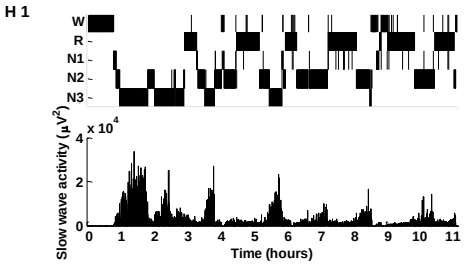
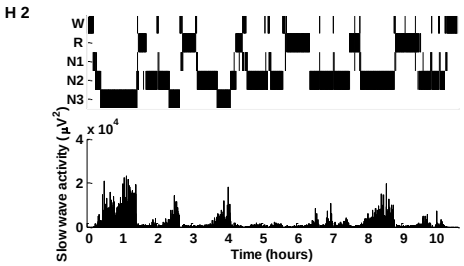
Supplementary material

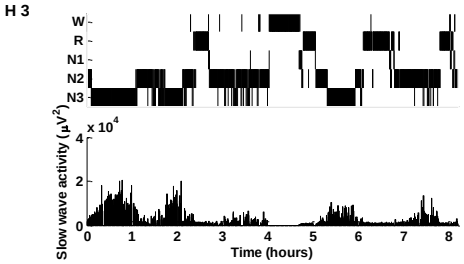
Supplementary Table 3 Medication of patients with disorders of consciousness, daily dosage

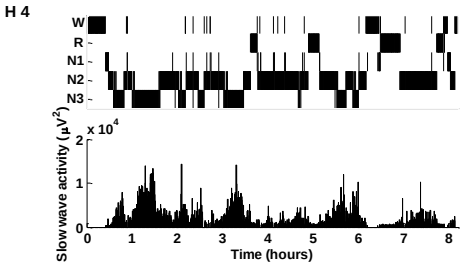
DOC 1, 4 years	DOC 2, 7 years	DOC 3, 12 years	DOC 4, 13 years	DOC 5, 14 years
S 1: Enalapril: 2.5 mg Macrogol: 7 g Esomeprazole: 20 mg Atenolol: 37.5 mg Diazepam: 7.5 mg Ondansetron: 2 mg Baclofen pump: 200 mcg	S 1: Omprazole: 20 mg Aspirin: 100 mg Paracetamol: 1500 mg Levothyroxine: 50 mcg Hydrocortison: 10 mg Potassium: 90 mmol Levetiracetam: 1600 mg Baclofen orally: 20 mg Macrogol: 17.5 g Desmopressin: 2.5 mcg Tetrabenazine: 25 mg	S 1 Clonidine: 75 mcg Levetiracetam: 1500 mg Insulin detemir: 16 Us Baclofen orally: 27.5 mg Melatonin: 5 mg Movicol: 1 Sachet Esomeprazole: 20 mg Pirazetam:15 ml (33%) Insulin aspart: 20 Us Levomepromazine: 37.5 mg Diazepam: 15 mg S 2: Clonidine: 75 mcg Levetiracetam: 1500 mg Insulin detemir: 11 Us Baclofen orally: 50 mg Melatonin: 5 mg Movicol: 0.5 Sachet Esomeprazole: 20 mg Insulin aspart: 14 Us Levomepromazine: 37.5 mg Sertraline: 50 mg Diazepam: 8 mg	S 1: Enoxaparine sodium: 40 mg Clonidine: 250 mcg Amoxicillin/clavulanic acid: 2000 mg Baclofen orally: 160 mg Macrogol: 14 g Esomeprazole: 40 mg Diazepam: 8 mg Tizanidine: 6 mg Tetrabenazine: 150 mg S 2: Baclofen orally: 90 mg Macrogol: 21 g Esomeprazole: 40 mg Diazepam: 3 mg Enoxaparine sodium: 40 mg Tetrabenazine: 150 mg	S 1: Enoxaparine sodium: 40 mg Clonidine: 225 mcg Paracetamol: 1000 mg Propranolol: 90 mg Baclofen orally: 12.5 mg Macrogol: 7 g Melatonin: 7.5 mg Esomeprazole: 20 mg Lorazepam: 2 mg S 2: Enoxaparine sodium: 40 mg Clonidine: 300 mcg Baclofen orally: 45 mg Macrogol: 14 g Solidago: 4 gtts

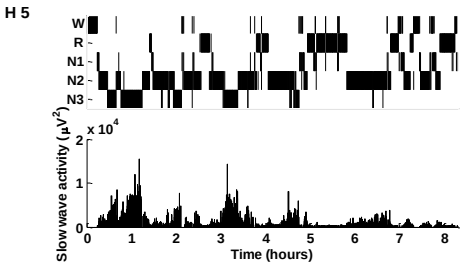
S1 = sleep recording first night; S2 = sleep recording second night; gtts = drops; Us = units

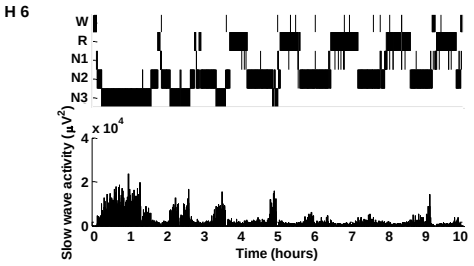

H 1
W
R
N1
N2
N3
x 10^4
Slow wave activity (μV²)
Time (hours)

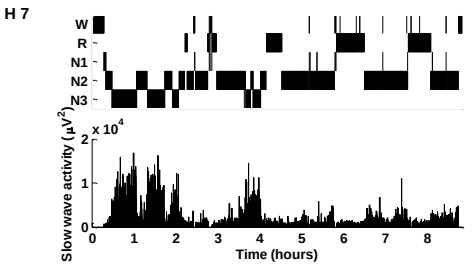

H 2
W
R
N1
N2
N3
x 10^4
Slow wave activity (μV²)
Time (hours)

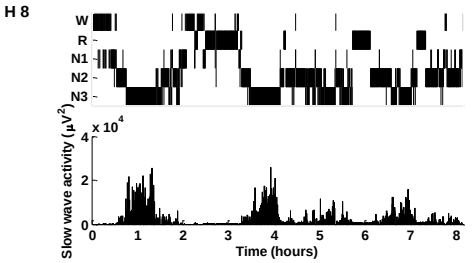

H 3
W
R
N1
N2
N3
x 10^4
Slow wave activity (μV²)
Time (hours)

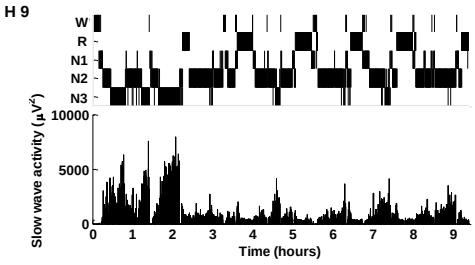

H 4
W
R
N1
N2
N3
x 10^4
Slow wave activity (μV²)
Time (hours)

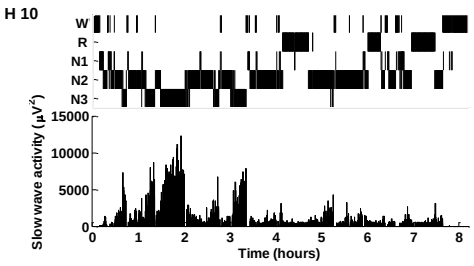

H 5
W
R
N1
N2
N3
x 10^4
Slow wave activity (μV²)
Time (hours)

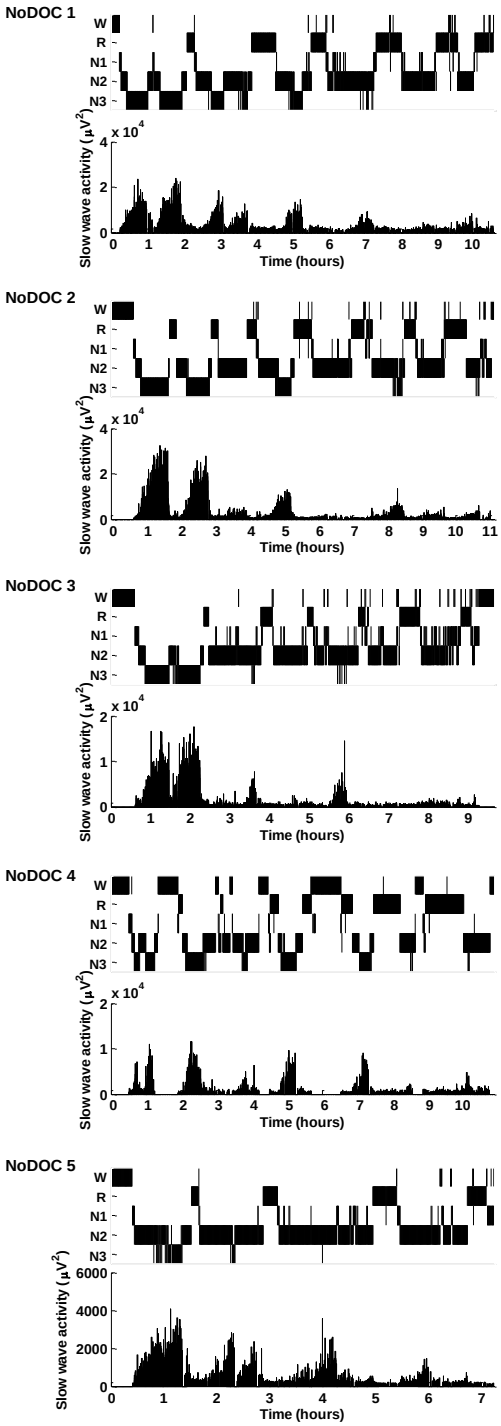

H 6
W
R
N1
N2
N3
x 10^4
Slow wave activity (μV²)
Time (hours)


H 7
W
R
N1
N2
N3
x 10^4
Slow wave activity (μV²)
Time (hours)

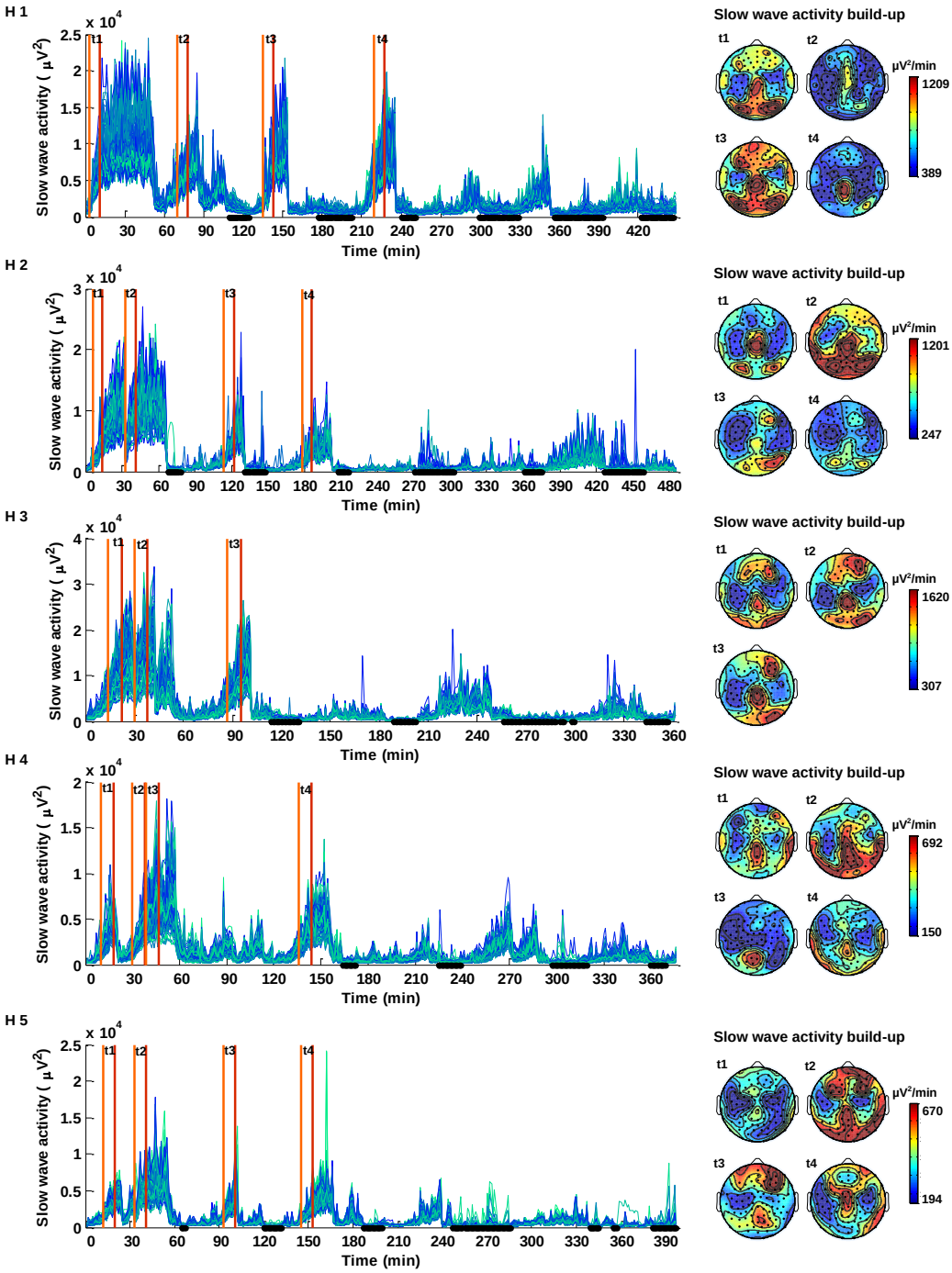

H 8
W
R
N1
N2
N3
x 10^4
Slow wave activity (μV²)
Time (hours)

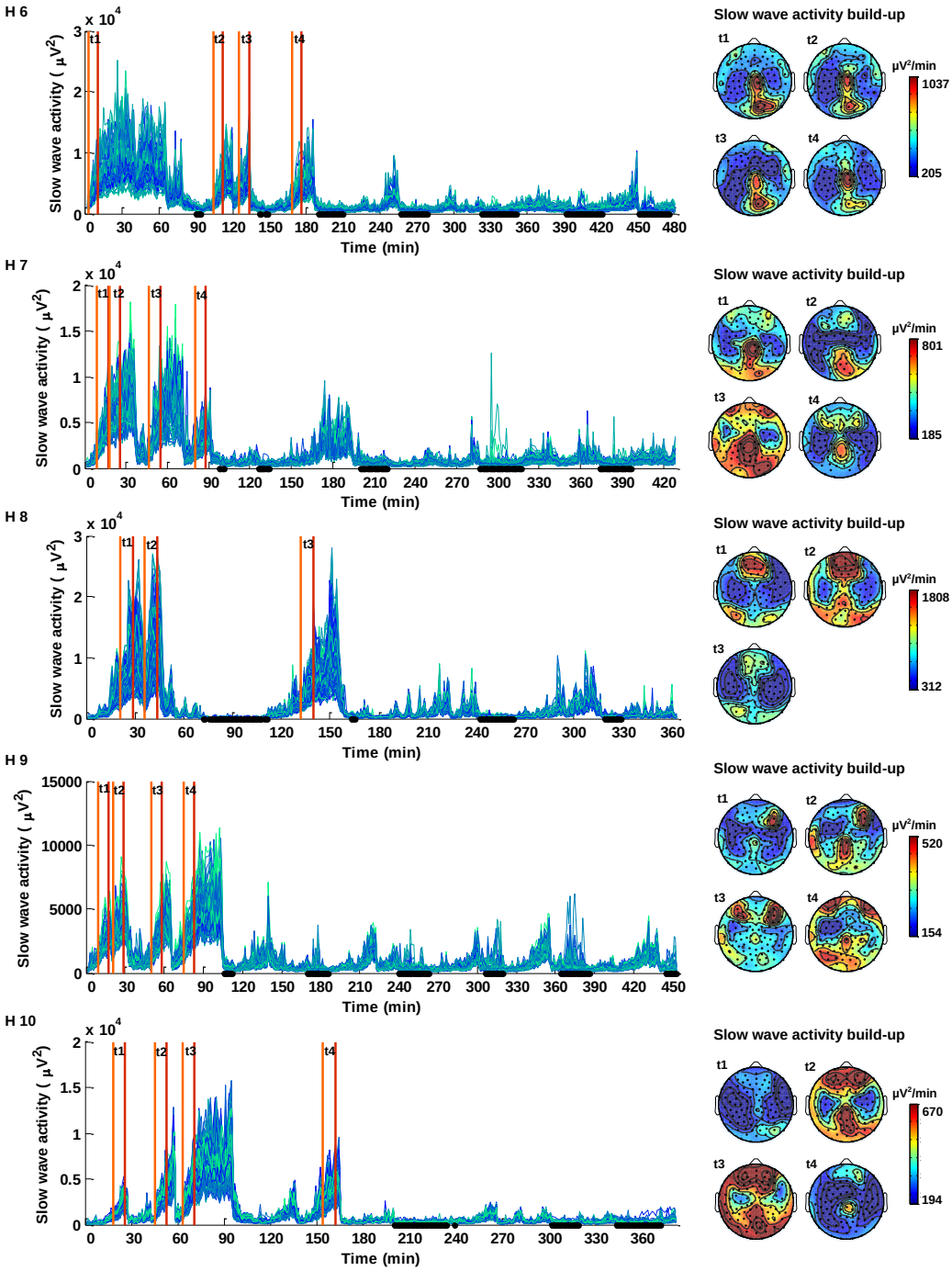

H 9
W
R
N1
N2
N3
Slow wave activity (μV²)
Time (hours)

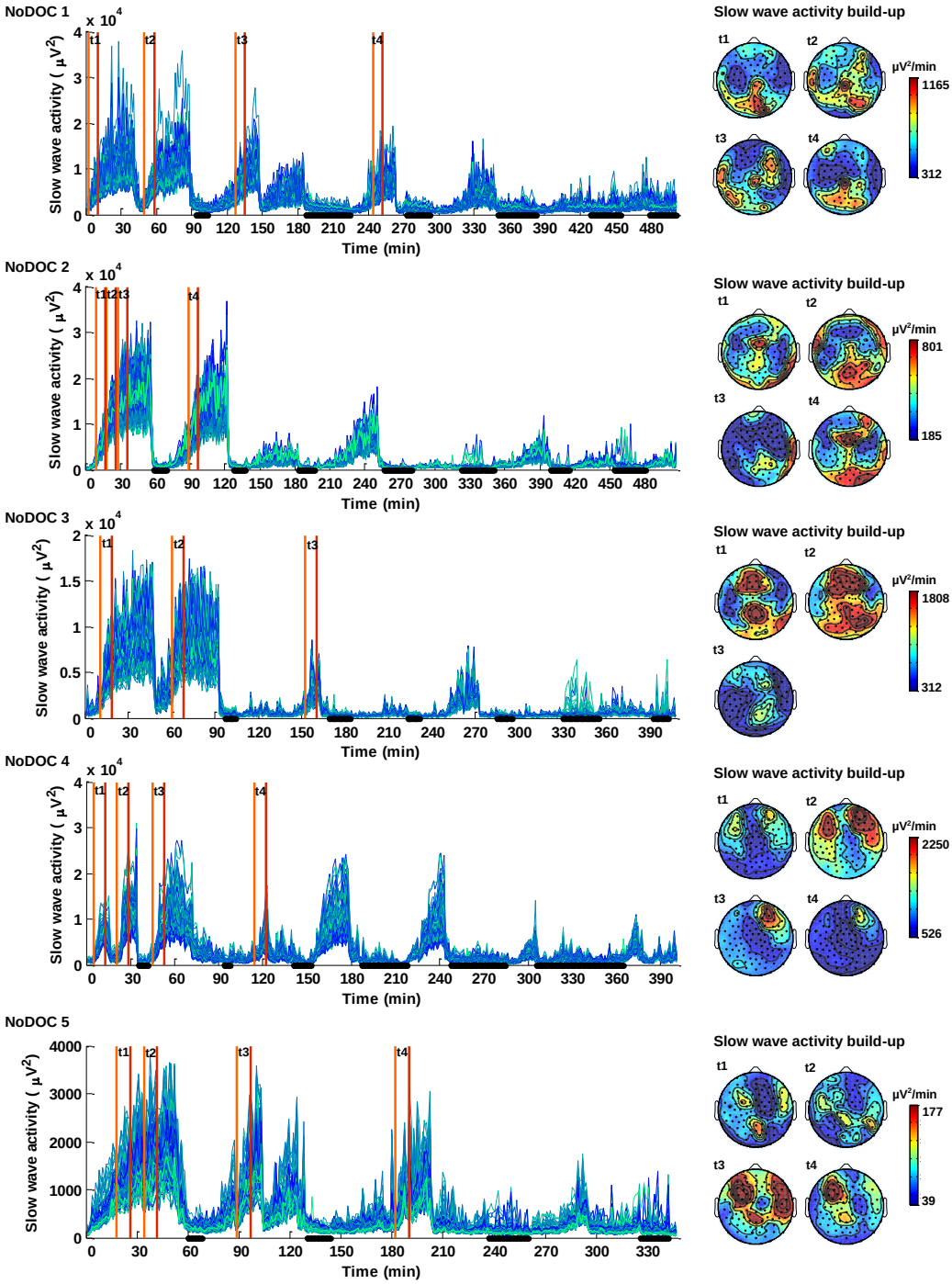

H 10
W
R
N1
N2
N3
Slow wave activity (μV²)
Time (hours)



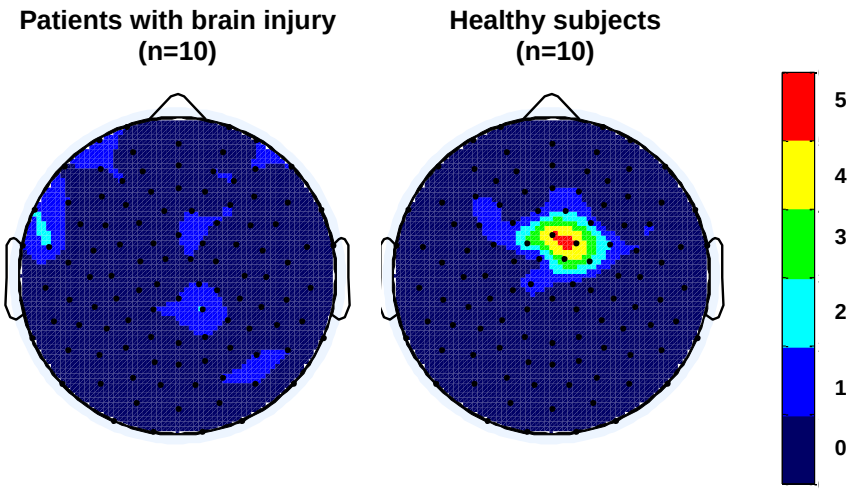
Supplementary Figure 1 Hypnogram and slow wave activity (SWA) time course across the night for ten healthy subjects (H) and five patients with acquired brain injury but without DOC (NoDOC). The sleep scoring includes wake (W), rapid eye movement sleep (R), NREM sleep stages N1, N2 and N3. SWA is shown for a single central derivation.


H 1
x 10^4
Slow wave activity (µV²)
t1
t2
t3
t4
Time (min)
Slow wave activity build-up
µV²/min
1209
389
H 2
1201
247
H 3
1620
307
H 4
692
150
H 5
670
194


H 6
x 10^4
Slow wave activity (µV²)
t1
t2
t3
t4
Time (min)
Slow wave activity build-up
µV²/min
1037
205
H 7
x 10^4
Slow wave activity (µV²)
Time (min)
Slow wave activity build-up
µV²/min
801
185
H 8
x 10^4
Slow wave activity (µV²)
Time (min)
Slow wave activity build-up
µV²/min
1808
312
H 9
Slow wave activity (µV²)
Time (min)
Slow wave activity build-up
µV²/min
520
154
H 10
x 10^4
Slow wave activity (µV²)
Time (min)
Slow wave activity build-up
µV²/min
670
194



Supplementary Figure 2 SWA trajectory and scalp distribution of SWA build-up for ten healthy subjects (H) and five patients with acquired brain injury but without DOC (NoDOC). SWA is shown across 1 min epochs. Each line represents the SWA time course for one electrode. Only artefact free sleep epochs were included. Black dots on the bottom line indicate epochs of REM sleep. Vertical lines in orange and magenta mark beginning and end of the four 9 min episodes of maximal SWA build-up. topographical plots (right) show the distribution of the SWA build-up for the four determined episodes. Colour coding was individually scaled in order to optimize the visualization of topographical patterns (maximal values in red, minimal values in blue).



Supplementary Figure 3 Frequency distribution of electrode clusters across patients with acquired brain injury and healthy subjects. The colours indicate the number of subjects in which a specific electrode is part of the maximal ratio cluster (relative SWA divided by relative SWA build-up).

3. DISCUSSION

The review article provided an overview of methods applied in pediatric sleep research and sleep medicine. In the first part of our research work, we investigated sleep in typically developing children and adolescents and focused on 1) state- and trait-like aspects in longitudinal measurements of topographical SWA and 2) the effect of a specific learning experience on sleep SWA. In a second part, we investigated sleep in children and adolescents with acquired brain injury, more specifically, 3) the relationship between SWA and lesion-related neural plasticity and 4) alterations in topographical SWA, in patients with DOC. In the following sections, our results and potential further research questions will be discussed.

3.1. Longitudinal measurements of topographical SWA in the course of development: state- and trait-like aspects

From late childhood to adolescence (i.e., from 10 to 15 years of age) within-subject topographical SWA increased over frontal brain areas and decreased over central brain areas. This state-like aspect is in line with the previously reported age-dependent differences in SWA topography, in a cross-sectional study (Kurth et al., 2010a). Moreover, the central decrease in topographical SWA was associated with performance in a reaching task (i.e., reduced movement variability). This finding again confirms the results from a cross-sectional study, which reported a link between maturational changes in SWA and the improvement of specific skills (Kurth et al., 2012).

However, individual characteristics in the topographical pattern of SWA were surprisingly consistent across measurements. Each subject showed a persistent individual expression of the maturational pattern. A previous longitudinal study in developing adolescents found another state-like aspect of the sleep EEG: the profile of the EEG power spectrum during non-REM sleep. The results showed that despite age-related changes in EEG amplitude, individual profiles of the EEG power spectrum are largely preserved across measurements (Tarokh et al., 2011a). These individual profiles are supposed to be genetically determined as indicated by twin studies in adults (De Gennaro et al., 2008; Landolt, 2011). It is likely that similar to the EEG power spectrum profile, trait-like aspects of the topographical distribution of SWA are also determined by genetic factors.

Altogether, these results indicate a considerable variability in the topographical distribution of SWA within typically developing children and adolescents. This variability

should be kept in mind when performing group comparisons, especially if groups are small. Differences in the state-like aspect of SWA topography could partially mask state-like differences and weaken the results.

3.2. The effect of a specific learning experience on sleep SWA: comparing children, adolescents and adults

After a visuomotor learning task, children, adolescents and adults showed a local increase in SWA over right parietal brain areas, which are known to be involved in task performance (Ghilardi et al., 2000). This result is in line with previous findings in adults (Huber et al., 2004). New learning experiences have been associated with changes in synaptic density and strength (for a review see (Fu and Zuo, 2011)). An increase in synaptic density and strength in turn, is known to increase SWA (Esser et al., 2007; Dash et al., 2009; Vyazovskiy et al., 2009). Thus, we assume that the local increase in parietal SWA reflects an increase in synaptic density and/or strength that was induced by visuomotor learning. Interestingly, the experience-dependent increase in SWA was most prominent in children when compared to adolescents and adults. This finding suggests a higher neuronal sensitivity to visuomotor learning in children, which would fit the concept of critical periods in the course of development (Hensch, 2004; Thomas and Johnson, 2008). Previous MRI studies could show that age at the time of skills acquisition determines the degree of structural changes in the brain. For instance, adults who had learned a second language during early childhood showed more pronounced neuronal changes than those who had learned a second language later (Mechelli et al., 2004). Similar results were shown for early musical training (Steele et al., 2013).

Our results showed a strong correlation between the local increase of SWA over right parietal areas and cortical gray matter volume in the same brain areas. These findings suggest that the more prominent SWA increase in children might be due to a higher amount of synapses involved in the learning-experience. Since the elimination of synapses in the course of brain maturation varies across different cortical regions (Giedd, 2004), time windows of maximal experience-dependent plasticity most likely are region-specific. An estimation of these time windows could have implications for education as well as for neurorehabilitation.

3.3. Sleep SWA and plasticity in children and adolescents with ABI

In children and adolescents with ABI, SWA was altered when compared to typically developing children and adolescents of the same age. Interestingly, these alterations seemed

to depend on nature, severity and site of the lesion, in particular for patients with stroke and severe TBI. In patients with unilateral stroke we found a local reduction in the topographical SWA over lesion areas. This might reflect neuronal death and impaired region-specific brain network function. After unilateral stroke patients also showed local increases in topographical SWA, mainly over peri-lesional and contralateral brain areas. Those areas are known to be involved in neural reorganization and functional recovery after stroke (Grefkes and Ward, 2014). Thus, we suggest that locally increased SWA may indicate "hyperplastic" brain areas involved in brain network restoration and/or recruitment of alternative networks.

In patients with severe TBI, alterations in topographical SWA were strikingly similar. All patients showed a reduction over the midline and an increase over lateral brain areas. This pattern is likely to reflect a common network dysfunction caused by axonal lesions, since none of the patients in this patient group showed cortical damages over the midline.

In a next step, longitudinal measurements in patients with ABI could assess plastic changes in the course of rehabilitation and link neural reorganization to function recovery.

3.3.1. Longitudinal measurements in the course of rehabilitation: investigating neural reorganization and function recovery

Preliminary results in a subgroup of five patients with hand motor deficits indicate that parietal brain areas might play an important role in hand motor function recovery (Figure 4). We analyzed two sleep measurements, one at the beginning of rehabilitation and one at a later time point (time interval 2 to 6 month). Hand motor performance was assessed in patients as soon as they were able to perform a test used in clinical routine (i.e., box and block test; (Mathiowetz et al., 1985)). Assessments were repeated in the course of rehabilitation to quantify progress.

Patients with a strong increase in parietal SWA from the first to the second measurement, showed a fast recovery of hand motor function within the time between the two sleep measurements. Patients who only slightly increased parietal SWA showed a slow recovery (i.e., patients were able to perform the test only after the second sleep measurement). One patient did not increase parietal SWA at all. This patient also did not recover any hand motor function (i.e., the patient was unable to perform the test to the end of rehabilitation therapy).

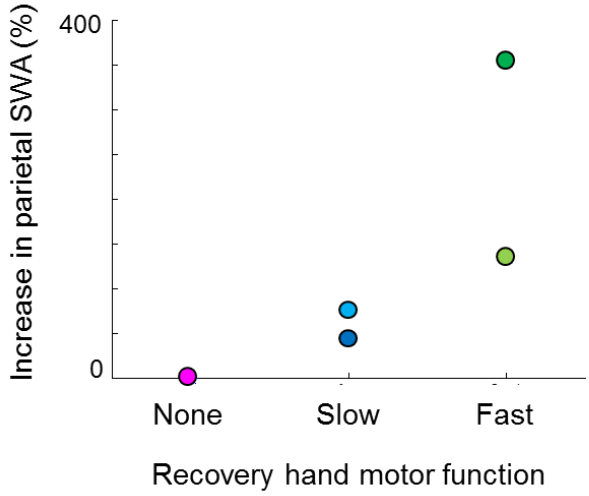


Figure 4 Increase in parietal slow wave activity (SWA) and recovery in hand motor function in five patients (different colors for individual patients).

In healthy adults, the posterior parietal cortex has been linked to hand motor skills such as reaching, grasping and the use of objects or tools (Vingerhoets, 2014). A longitudinal study in adult stroke patients found that lesions in the parietal cortex impaired recovery of dexterous hand function (Abela et al., 2012). In chronic stroke patients, structural parieto-frontal connectivity was associated with hand motor function (Schulz et al., 2015).
In line with these findings, our results point out that parietal brain areas might be critically involved in the reorganization of neural networks underlying hand motor function.

Further research could specifically investigate children with ABI who recovered hand motor function. For instance, fMRI measurements during a hand motor task might provide informative insights into recruiting pattern of reorganized networks.

3.3.2. Does age matter?

There is an ongoing debate about whether ABI during development results in particularly good or particularly poor outcome. Good outcomes would be due to 'early plasticity': maturational processes have already been initiated, however, functional specificity is not yet fully established. Thus, functions from damaged areas could be taken over from undamaged connected areas. Bad outcomes would be due to 'early vulnerability', caused by the absence of a 'blueprint' that could guide recovery in brain areas in which maturational processes have not yet been initiated (Pascual-Leone et al., 2005). Anderson et al. (Anderson et al., 2011) propose a 'recovery continuum' between the opposite extremes 'early plasticity' and 'early

vulnerability'. According to the authors, an individual child's recovery would be situated along this continuum depending on several factors (i.e., age, site and size of the injury, nature of the injury and environmental factors like family support).

At this point, our results cannot contribute to a better understanding of the dynamics of recovery. Our patient group was too heterogeneous and too small to allow any assumption. To pursue this objective we would need measurements from multiple patients of different ages, with similar brain injuries. In addition, long-term studies could assess neurological deficits in the further course of development and investigate possible alterations in brain maturation after ABI. It has been reported that some pediatric patients with ABI seem to 'grow into new deficits' over time (Anderson et al., 2011).

3.3.3. Can sleep measurements assess the effectiveness of specific therapies?

Another future benefit of longitudinal sleep measurements in patients with ABI might be the possibility to assess effects of specific rehabilitative interventions on brain function. This has been done in a study investigating changes in sleep SWA after a specific speech therapy in patients with aphasia after stroke (Sarasso et al., 2014). The applied therapy (IMITATE, (Lee et al., 2010)) was developed based on a proposed neurophysiological concept of rehabilitation therapy (Small et al., 2013). According to this concept, therapy should stimulate neural plasticity to facilitate network reorganization. This goal can be pursued directly via training of the impaired function or indirectly via activities known to share a common neural network with the impaired function. In the case of aphasia, it is known that speech production and speech observation activate common neural networks (Skipper et al., 2005). IMITATE now aims at activating this network by observing and imitating speech (6 weeks protocol with 90min per day). In the previously mentioned sleep study, patients were exposed to a single session of IMITATE therapy. In the night after the therapy session, sleep SWA was increased over brain areas predicted by the therapy concept (Sarasso et al., 2014). Although further studies should assess the effect of the entire therapy protocol, these first results indicate that the proposed therapy indeed induces synaptic changes in the targeted neural network. Likewise, other indirect training effects on specific functions could be investigated by means of sleep measurements, for example effects of musical training on language processing (Chobert et al., 2014) or transfer effects of working memory training on other cognitive functions (Bigorra et al., 2015; Söderqvist and Bergman Nutley, 2015).

Another novel approach for rehabilitation therapy is brain stimulation. Methods such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS)

offer the possibility of directly targeting brain structures to facilitate or inhibit their activity. Several applications for rehabilitation have been proposed (Chung and Lo, 2015; Page et al., 2015). These still conversely discussed approaches could be evaluated by means of sleep measurements. Interestingly, a recent neuropharmacological study in healthy adults could show that enhancing N-methyl-D-aspartate receptor (NMDAR) activity boosts experience-dependent plasticity and increases learning performance (Forsyth et al., 2015). Combining pharamacotherapy with rehabilitation therapy might be a promising approach to promote recovery. Again induced plastic changes could be assessed by means of sleep recordings.

3.3.4. Is sleep essential for recovery?

Animal studies could show that sleep deprivation prior to stroke have a neuroprotective effect, which is associated with better sensorymotor and motor recovery (Moldovan et al., 2010; Cam et al., 2013). It has been suggested that sleep rebound (i.e., increase in the amount of sleep after sleep deprivation) may be causally related to neuroprotection (Cam et al., 2013). Accordingly, sleep deprivation in the acute phase after stroke was found to have detrimental effects (Gao et al., 2010; Zunzunegui et al., 2011).

In humans, sleep disturbances following stroke have been related to poor outcome (Hermann and Bassetti, 2009). Studies investigating sleep and motor learning in patients with stroke demonstrated that sleep promotes offline motor learning (Siengsukon and Boyd, 2008; Siengsukon and Boyd, 2009a; Siengsukon and Boyd, 2009b).

In healthy subjects, sleep-dependent offline learning has also been reported in the context of daytime naps (Mednick et al., 2003; Backhaus and Junghanns, 2006; Nishida and Walker, 2007). Furthermore, it has been shown that a nap, immediately after training, reduces the susceptibility to interference by a subsequent learning-experience (Korman et al., 2007). This could have implications for rehabilitation therapy. Implementing naps between therapy sessions might consolidate training effects.

The beneficial effect of sleep on performance has been related to SWA (Marshall and Born, 2007; Diekelmann and Born, 2010; Rasch and Born, 2013). To further determine the causal role of SWA in performance improvement several studies have manipulated SWA. For instance, selective slow wave suppression by means of acoustic stimulation, prevented over night performance improvement without affecting overall sleep time and efficiency (Landsness et al., 2009). A study enhancing SWA pharmacologically reported an improvement in cognitive performance (Walsh et al., 2006). In another study, the application of tDCS at 0.75 Hz during slow wave sleep was found to increase activity in the slow

oscillation frequency band (<1Hz). This increase was associated with improved memory performance (Marshall et al., 2006). Also studies using specifically timed acoustic stimulation could increase slow oscillation activity and improve memory performance (Ngo et al., 2013a; Ngo et al., 2013b). Finally, a study stimulating the vestibular system could demonstrate that a gentle rocking of the bed increases SWA during a nap (Bayer et al., 2011).

In the future, such novel approaches to boost SWA could be applied in the clinical setting. Studies should then evaluate whether they have a beneficial effect on recovery.

3.4. Sleep regulation in children and adolescents with DOC

In a small patient group of children and adolescents with DOC we investigated alterations in sleep regulation using the build-up of SWA. Similar to SWA itself, the build-up of SWA is a use-dependent measure reflecting brain activity during previous wakefulness. We decided to use the build-up of SWA based on the assumption that in patients with severe ABI, SWA might be confounded by the presence of lesion-related slow-oscillations arising as a result of cortical deafferentation (Steriade et al., 1993c; Lemieux et al., 2014). In contrast to sleep slow waves we hypothesized these pathophysiological slow oscillations to be independent of sleep regulatory processes.

Patients with DOC showed a widespread reduction in the amount of SWA build-up, when compared to both, healthy subjects and patients with ABI without DOC. This overall decrease in SWA regulation indicates a global reduction of functional brain activity in patients with DOC. The topographical distribution of the SWA build-up revealed local differences. Patients with DOC showed a reduced topographical SWA build-up over parietal brain areas and over a smaller frontal brain area when compared to the two other groups. Noteworthy, these findings correspond to the results from our analysis on the single-subject level (see 2.4. Sleep slow wave activity: towards a new marker for neural plasticity after acquired brain injury, patients 1-3,9,10). Since no patient had a local damage in parietal brain areas, the parietal reduction in the SWA build-up does not simply reflect brain damage but might rather indicate a disorder-specific alteration in brain network function. Moreover, within our patient group, the amount of parietal SWA build-up was related to outcome.

Our results are in agreement with a recent high-density EEG study that found resting state spectral power measures over frontal and parietal brain regions to be sensitive indices of consciousness in adults (Sitt et al., 2014). Also functional MRI and PET studies identified the frontoparietal network to be critically involved in DOC (Laureys and Schiff, 2012; Crone et

al., 2014). Thus, the local reduction of SWA regulation over parietal brain areas might indeed reflect impaired brain activity during previous wakefulness.

In the context of the proposed mesocircuit model of consciousness (Giacino et al., 2014), the reduced SWA regulation over parietal brain areas could be explained by a reduced function of the thalamus and/or impaired functional thalamocortical connectivity. The insufficient synaptic input would result in a reduced activation of the parietal cortex and thereby prevent local processing and long-distance functional corticocortical connectivity. In the future, local aspects of sleep regulation in adult patients with DOC would be worthwhile. First, patient numbers in the adult population are higher than in the pediatric population and therefore results might provide an even clearer picture of network dysfunction. Second, comparing pediatric and adult patients could reveal different patterns of thalamocortical dysfunction depending to the maturational state of the brain.

Additional insights into thalamocortical connectivity might be provided by the analysis of sleep spindles. Sleep spindles in the EEG are waxing and waning oscillations between 12 and 15 Hz. They are generated in the thalamus and modulated by thalamocortical loops (Steriade, 2003). In patients with schizophrenia, a few studies have investigated the topographical distribution of sleep spindle activity by means of high-density EEG. Adult patients as well as adolescents with early onset schizophrenia showed a reduction of sleep spindles in centroparietal and temporal brain areas (Ferrarelli et al., 2007; Ferrarelli et al., 2010; Tesler et al., 2015). Moreover, these deficits were related to the severity of positive symptoms (Ferrarelli et al., 2010; Tesler et al., 2015). These findings suggest that topographical alterations in the activity of sleep spindles reflect a disorder-specific dysfunction of thalamocortical networks. Performing such analysis in patients with DOC could provide region-specific information about impaired thalamocortical connectivity.

3.4.1. The clinical value of sleep measurements in patients with DOC

While functional MRI and PET may not always be available in the clinical setting, EEG measurements can easily be performed at the bedside. Another advantage of EEG is the possibility of long-duration measurements including sleep. Such long-duration measurements are especially convenient, when assessing patients with DOC, as these patients typically show frequent fluctuations in the level of arousal (Forgacs et al., 2014). Several studies could show that assessing sleep structure integrity (i.e., the presence of characteristic features of non-REM sleep such as sleep spindles and slow waves, and the presence of different sleep stages including REM sleep) might provide valuable complementary diagnostic information and/or

predict outcome (Cheliout-Heraut et al., 2002; Landsness et al., 2011; Cologan et al., 2012; Malinowska et al., 2013; de Biase et al., 2014; Kang et al., 2014; Rossi Sebastiano et al., 2014; Aricò et al., 2015; Arnaldi et al., 2015; Avantaggiato et al., 2015). One study proposed to use long-term EEG measurements including sleep to screen for patients who are likely to have preserved unrecognized cognitive abilities despite a lack of behavioral responses (Forgacs et al., 2014). A recent study proposed to assess effects of repetitive TMS (rTMS) on SWA during subsequent sleep (Pisani et al., 2015). It is known that in healthy subjects SWA is locally increased after rTMS (Huber et al., 2007). Such an increase was found in MCS patients but not in VS/UWS patients. The preservation of experience-dependent plasticity could indicate residual network function related to consciousness.

One of our patients did not show any improvement in the level of consciousness. In this patient, we found a residual regulation of SWA over right occipital (visual) brain areas (Figure 5). Interestingly, this fitted the patient's clinical picture. When assessed with the CRS-R, this patient only scored on a single item indicating minimal consciousness: unilateral visual pursuit in the left visual field. Thus, in chronic patients with DOC, areas of residual SWA regulation might indicate preserved functions.

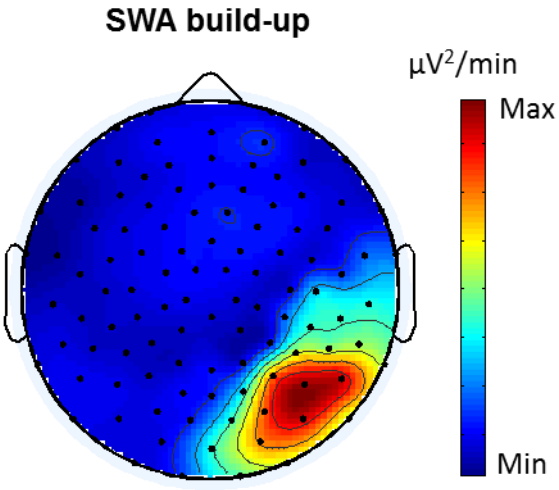


Figure 5 Slow wave activity (SWA) build-up topography of a 4 years old patient (see 2.4. High-density electroencephalographic recordings during sleep in children with disorders of consciousness, patient1). In red brain areas showing a residual SWA build-up.

In adult patients a few treatment interventions were shown to have beneficial effect on consciousness (Giacino et al., 2014). For instance the administration of amantadine in subacute patients accelerates the recovery process (Giacino et al., 2012b). The administration

of zolpidem seems to be selectively effective. A few patients respond to the medication whereas most of them show no improvements (e.g., (Brefel-Courbon et al., 2007; Shames and Ring, 2008; Singh et al., 2008; Whyte and Myers, 2009; Thonnard et al., 2013)). Central thalamic deep brain could promote recovery in chronic patients (Schiff et al., 2007; Giacino et al., 2012a) and a recent study applying tDCS transiently improved signs of consciousness in MCS patients (Thibaut et al., 2014). In the future, sleep measurements pre- and post-treatment could provide insights into induced changes in brain activity and improve our understanding of neural mechanisms underlying consciousness.

4. OVERALL CONCLUSIONS

We investigated sleep and plasticity in the context of healthy development and after acquired brain injury. Longitudinal sleep measurements in the course of adolescence point out that changes in topographical SWA likely reflect brain maturation. Further we investigated effects of a specific learning task on SWA and could show that experience-dependent plasticity induces local changes in topographical SWA. Since SWA seems to be a sensitive method to assess plastic processes in the healthy brain, we expanded its use to investigate plasticity after ABI. We found that indeed, the SWA topography in children and adolescents with ABI shows lesion-related alterations. Our approach to detect alterations on the single-subject level allowed us to determine individual areas of impaired neural function and/or 'hyperplastic' areas presumably involved in network reorganization. The localization of such 'hyperplastic' brain areas could provide a basis for novel therapeutic interventions like for instance brain stimulation. Targeting orchestrating reorganization areas might boost plasticity in an entire network.

In patients with DOC, we used the regulation of SWA as an indirect measure for brain activity during previous wakefulness. We found a disorder-specific reduction over parietal brain areas. Residual parietal SWA regulation might provide additional diagnostic as well as prognostic information. On the one hand, this measure might reflect functional network integrity related to consciousness levels, on the other hand, it might indicate plastic capacity related to outcome.

Our studies were the first to investigate neural plasticity in children and adolescents with ABI by means of high-density EEG recordings during sleep. Such recordings are easy to apply in the clinical setting, also in critically ill and non-cooperative patients. Overall, our approach appears to provide sensitive markers for individual neural reorganization and novel

neural correlates for disorders of consciousness. Functional measurements of brain activity during sleep could complement behavioral assessments and structural information provided by MRI. Improving our understanding of neural plasticity after ABI could not only assist clinical diagnosis and prognosis but may also guide the development of novel therapeutic interventions.

Longitudinal measurements could assess plastic changes in the course of rehabilitation and link neural reorganization to function recovery. Preliminary results in a subgroup of five patients with hand motor deficits indicate that parietal brain areas might be critically involved in the reorganization of neural networks underlying hand motor function. Moreover, longitudinal sleep measurements might provide the possibility to assess effects of specific rehabilitative interventions on neurophysiology.

Finally, the active role of sleep in the recovery process could be investigated by modulating SWA during sleep. Several non-pharmacological approaches enhancing sleep SWA have been successfully applied in healthy subjects. Future studies should evaluate whether such approaches could be used to promote recovery in the context of rehabilitation therapy.

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CURRICULUM VITAE

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Academic degrees

11.2010	M Sc, University of Zurich, Switzerland
06.2000	Diploma in Primary education, Teacher Training College Chur, Switzerland

Professional experience

01.2011 – present	Ph.D. Student in the groups of Dr. Reto Huber, Pediatric Sleep Research, University Children's Hospital Zurich, University of Zurich, Switzerland and PD Dr. Huub van Hedel, Pediatric Rehab Research Group, Rehabilitation Centre Affoltern am Albis, University Children's Hospital Zurich, University of Zurich, Switzerland
01.2009 – 06.2009	Internship, Psychiatric clinic „Littenheid", Switzerland
08.2000 – 07.2005	Teacher, Primary school in Zurich, Switzerland

Grants

08.2013 – 07.2015	Sleep measurements in children and adolescents with acquired brain injury: a new approach to investigate neuronal reorganization in the course of rehabilitation. Anna-Mueller-Grocholski Foundation

Awards

2013	Best poster award: 4th annual scientific retreat of the Children's Research Centre, University Children's Hospital Zurich, Switzerland
2014	Best oral presentation award: 5th annual scientific retreat of the Children's Research Centre, University Children's Hospital Zurich, Switzerland
2015	Anna-Müller-Grocholski-Preis: awarded by the Swiss Academy of Childhood Disability

Publication list

2013	Lee GM, Fattinger S, Mouthon AL, Noirhomme Q, Huber R. *Electroencephalogram approximate entropy influenced by both age and sleep.* Front Neuroinform. 2013 Dec 5;7:33.201
2014	Wilhelm I, Kurth S, Ringli M, Mouthon AL, Buchmann A, Geiger A, Jenni OG, Huber R. *Sleep slow-wave activity reveals developmental changes in experience-dependent plasticity.* J Neurosci. 2014 Sep 10;34(37):12568-75.
2015	Mouthon AL, Huber R. Methods in pediatric sleep research and sleep medicine. Neuropediatrics. 2015 Jun;46(3):159-70.
Under review	Mouthon AL, van Hedel HA, Meyer-Heim A, Kurth S, Ringli M, Pugin F, Huber R. *High-density electroencephalographic recordings during sleep in children with disorders of consciousness*.
Submitted	Mouthon AL, van Hedel HA, Meyer-Heim A, Kurth S, Ringli M, Pugin F, Huber R. *Sleep slow wave activity: towards a new marker for neural plasticity after acquired brain injury*
Submitted	Lustenberger C, Mouthon AL, Tesler N, Kurth S, Ringli M , Buchmann A Jenni OG, Huber R. *Developmental trajectories of EEG sleep slow wave activity as a marker for brain and motor skill development during adolescence*.
In preparation	Mouthon AL, van Hedel HA, Meyer-Heim A, Kurth S, Ringli M, Pugin F, Huber R. *Longitudinal changes in sleep slow wave activity provide novel insights into neural reorganization after acquired brain injury*.

Oral presentations and posters

2012 **Poster:** *The build-up of electroencephalographic sleep slow wave activity: towards a new marker for functional brain activity in children with acquired brain injury*. Annual scientific retreat of the Children's Research Centre, Universitäts Kinderspital Zürich, Au, Switzerland.

2012 **Poster**: *The build-up of electroencephalographic sleep slow wave activity: towards a new marker for functional activity in brain injured children*. Jahrestagung der Schweizerischen Gesellschaft für Neuropädiatrie, Bern, Switzerland.

2013 **Poster:** *The build-up of electroencephalographic sleep slow wave activity: towards a new marker for functional brain activity in children with acquired brain injury*. Annual meeting of the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology, Aarau, Switzerland.

2013 **Poster:** *Sleep after stroke in children: investigating the course of recovery*. Symposium Zentrum für Neurowissenschaften, Zürich, Switzerland.

2013 **Poster:** *Slow wave sleep in young stroke patients - investigating its relationship with behavioural performance*. Annual scientific retreat of the Children's Research Centre, Universitäts Kinderspital Zürich, Au, Switzerland.

2014 **Competitive selection for an oral presentation:** *Sleep in children during stroke recovery – a new approach to investigate brain reorganization and related functional outcome*. World Congress for Neurorehabilitation, Istanbul, Turkey.

2014 **Poster:** *The build-up of sleep slow wave activity in young patients with stroke - investigating alterations in the topographical distribution and longitudinal changes in the course of recovery*. Annual meeting of the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology, Luzern, Switzerland.

2014 **Poster:** *Longitudinal changes in the build-up of sleep slow wave activity in young patients recovering from acquired brain injury*. European Sleep Research Society Congress, Tallinn, Estonia.

2014 **Präsentation:** *Longitudinal changes in the build-up of sleep slow wave activity in young patients recovering from acquired brain injury*. Annual scientific retreat of the Children's Research Centre, Universitäts Kinderspital Zürich, Au, Switzerland.

2015 **Präsentation:** *Slow wave sleep regulation in children with disorders of consciousness: a marker reflecting the maturational stage of the brain and behavioural changes*. Symposium Sleep, Cognition and Consciousness, Zell am See, Austria.

2015 **Competitive selection for an oral presentation:** *The local regulation of sleep is related to behaviour in children with disorders of consciousness*. Annual meeting of the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology, Interlaken, Switzerland.

2015 **Poster:** *High density sleep EEG recordings in children with disorders of consciousness*. . Annual scientific retreat of the Children's Research Centre, Universitäts Kinderspital Zürich, Au, Switzerland.

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